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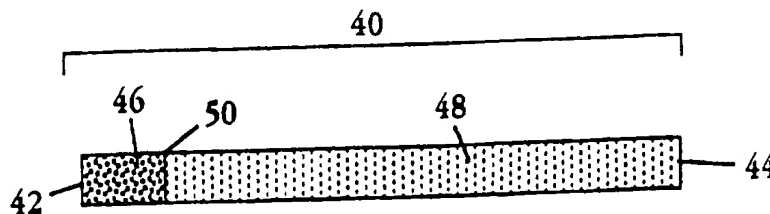
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(54) Title: HYBRID INTERFERON COMPOSITIONS AND METHODS OF USE

(57) Abstract

The present invention describes hybrid interferon fusion polypeptides formed of a first segment that contains the N-terminal amino acid sequence of an interferon-tau polypeptide, and a second segment that contains the C-terminal amino acid sequence of a non-tau interferon type I polypeptide. The two segments are joined in the region of a mature interferon polypeptide between about residues 8 and 37. Also described are nucleic acid sequences encoding such interferon fusion polypeptides, expression vectors containing such sequences, and therapeutic applications of the interferon fusion polypeptides. The therapeutic applications include antiviral and anticellular proliferation applications. One advantage of the interferon fusion polypeptides of the present invention is that they do not have cytotoxic side-effects when used to treat cells.

HYBRID INTERFERON COMPOSITIONS AND METHODS OF USE

Field of the Invention

The present invention relates to hybrid interferon proteins consisting of a region or regions derived from interferon-τ and a region or regions derived from another interferon.

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Background of the Invention

Conceptus membranes, or trophectoderm, of various mammals produce biochemical signals
that allow for the establishment and maintenance of pregnancy (Bazer, *et al.*, 1983). One such
protein, ovine trophoblast protein-one (oTP-1), was identified as a low molecular weight protein

secreted by sheep conceptuses between days 10 and 21 of pregnancy (Wilson, *et al.*, 1979; Bazer, *et al.*, 1986). The protein oTP-1 was shown to inhibit uterine secretion of prostaglandin F₂-alpha, which causes the corpus luteum on the ovary to undergo physiological and endocrinological demise in nonpregnant sheep (Bazer, *et al.*, 1986). Accordingly, oTP-1 has antiluteolytic biological
5   activity. The primary role of oTP-1 was assumed to be associated with the establishment of pregnancy.

oTP-1 was subsequently found to (i) exhibit limited homology (50-70%) with interferon alphas (IFNα) of various species (Imakawa, *et al.*, 1987), and (ii) bind to a Type I interferon receptor (Stewart, *et al.*, 1987). Despite some similarities with IFNα, oTP-1 has several features
10 that distinguish it from IFNα including the following: oTP-1's role in reproductive biochemistry (other interferons are not known to have any role in the biochemical regulation of reproductive cycles), oTP-1's cellular source -- trophoblast cells (IFNα is derived from lymphocyte cells), oTP-1's size -- 172 amino acids (IFNα is typically about 165-166 amino acids), and oTP-1 is weakly inducible by viruses (IFNα is highly inducible by viruses). The International Interferon Society
15 recognizes oTP-1 as belonging to an entirely new class of interferons which have been named interferon-tau (IFNτ). The Greek letter τ stands for trophoblast.

The interferons have been classified into two distinct groups: type I interferons, including IFNα, IFNβ, and IFNω (also known as IFNαII); and type II interferons, represented by IFNγ (reviewed by DeMaeyer, *et al.*, 1988). In humans, it is estimated that there are at least 17 IFNα
20 non-allelic genes, at least about 2 or 3 IFNβ non-allelic genes, and a single IFNγ gene.

IFNα's have been shown to inhibit various types of cellular proliferation. IFNα's are especially useful against hematologic malignancies such as hairy-cell leukemia (Quesada, *et al.*, 1984). Further, these proteins have also shown activity against multiple myeloma, chronic lymphocytic leukemia, low-grade lymphoma, Kaposi's sarcoma, chronic myelogenous leukemia,
25 renal-cell carcinoma, urinary bladder tumors and ovarian cancers (Bonnem, *et al.*, 1984; Oldham, 1985). The role of interferons and interferon receptors in the pathogenesis of certain autoimmune and inflammatory diseases has also been investigated (Benoit, *et al.*, 1993).

IFNα's are also useful against various types of viral infections (Finter, *et al.*, 1991). Alpha interferons have shown activity against human papillomavirus infection, Hepatitis B, and Hepatitis
30 C infections (Finter, *et al.*, 1991; Kashima, *et al.*, 1988; Dusheiko, *et al.*, 1986; Davis, *et al.*, 1989).

Significantly, however, the usefulness of IFNα's has been limited by their toxicity: use of interferons in the treatment of cancer and viral disease has resulted in serious side effects, such as fever, chills, anorexia, weight loss, and fatigue (Pontzer, *et al.*, 1991; Oldham, 1985). These

side effects often require (i) the interferon dosage to be reduced to levels that limit the effectiveness of treatment, or (ii) the removal of the patient from treatment. Such toxicity has reduced the usefulness of these potent antiviral and antiproliferative proteins in the treatment of debilitating human and animal diseases.

5   **Summary of the Invention**

In one aspect, the invention includes chimeric nucleic acid molecules encoding hybrid interferon fusion polypeptides. Each such molecule is formed of a 5' end segment that encodes the N-terminal amino acid sequence of an interferon-tau polypeptide, and a 3' end segment that encodes the C-terminal amino acid sequence of a non-tau interferon type I polypeptide. The two segments

10   are spliced in a region corresponding to the portion of a mature interferon polypeptide between about residues 8 and 37. Examples of non-tau interferon type I polypeptides include interferon alpha and interferon beta. In one embodiment, the 5' end segment further includes a leader sequence.

Examples of sequences encoded by the 5' end segment include these contained in a sequence

15   selected from the group consisting of SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:35, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53. In particular, sequences contained in SEQ ID NO:5, SEQ ID NO:15 or SEQ ID NO:35. The 5' end segment may also encode a consensus sequence from any of the above, or an internally-consistent sequence. Preferred embodiments are

20   where the sequence is derived from a human source, such as S⊤ ID NO:15 or SEQ ID NO:35.

The 3' end segment may encode, in various embodim⸲ .ts, an amino acid sequence derived from an interferon alpha 1, alpha 2, beta or omega. The 3' end segment may also encode a consensus sequence from any of the above, or an internally-consistent sequence. Preferred embodiments are where the sequence is derived from a human source, such as a human IFNα or human

25   IFNβ.

In a general embodiment, the two segments are spliced in a region corresponding to the portion of a mature interferon polypeptide between about residues 8 and 28. In another general embodiment, the two segments are spliced in a region corresponding to the portion of a mature interferon polypeptide between about residues 8 and 22. In yet another general embodiment, the

30   two segments are spliced in a region corresponding to the portion of a mature interferon polypeptide between about residues 8 and 16. In one preferred embodiment, the two segments are spliced at a location corresponding to residue 22 of a mature interferon polypeptide.

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In other general embodiments, the interferon-tau polypeptide may be a human or ruminant (e.g., ovine) interferon-tau polypeptide, and the non-tau interferon type I polypeptide may likewise be a human or non-human interferon type I polypeptide. In a preferred embodiment, the interferon-tau polypeptide is an ovine interferon-tau polypeptide, and the non-tau interferon type
5   I polypeptide is a human interferon type I polypeptide.

An exemplary chimeric nucleic acid molecule of the present invention is SEQ ID NO:54, with a 5'-end segment encoding amino acids 1-28 of human IFNtau (from SEQ ID NO:3) and a 3'-end segment encoding amino acids 29-167 of human IFNα (clone pIFN105; Genbank HUMIFNN Acc. M28585).

10   Any of the chimeric nucleic acid molecule described above may further includes a leader sequence.

In a related aspect, the invention includes a hybrid interferon fusion polypeptide formed of a first segment that contains the N-terminal amino acid sequence of an interferon-tau polypeptide, and a second segment that contains the C-terminal amino acid sequence of a non-tau interferon
15   type I polypeptide. The two segments are joined in the region of a mature interferon polypeptide between about residues 8 and 37. Specific embodiments are as described above for chimeric acid molecules. Interferon-α and interferon-β are examples of such non-tau type I interferons. Such hybrid fusions may be used to reduce the toxicity of the non-tau type I interferons when the interferons are used in pharmaceutical formulations or in therapeutic applications.

20   The first segment may have, in various embodiments, an amino acid sequence contained in a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53. Preferred embodiments are where the sequence corresponds to a human interferon tau, such as SEQ ID NO:15 or SEQ ID NO:35.

25   In a general embodiment, the two segments are joined in the region of a mature interferon polypeptide between about residues 8 and 28. In another embodiment, they are joined in the region of a mature interferon polypeptide between about residues 8 and 22. In still another embodiment, they are joined in the region of a mature interferon polypeptide between about residues 8 and 16.

30   In another aspect, the present invention includes an expression vector having a nucleic acid containing an open reading frame (ORF) that encodes a hybrid interferon fusion polypeptide, including the nucleic acid and polypeptide sequences described above. The vector further includes regulatory sequences effective to express the open reading frame in a host cell: such sequences may be endogenous (such as the normally occurring IFN leader sequences, or heterologous (such

as a secretory signal recognized in yeast, mammalian cell, insect cell, tissue culture or bacterial expression systems). In the expression vector, regulatory sequences may also include, 5' to said nucleic acid sequence, a promoter region and an ATG start codon in-frame with the hybrid interferon fusion polypeptide coding sequence (chimeric nucleic acid molecule), and 3' to said coding

5   sequence, a translation termination signal followed by a transcription termination signal. Further, the invention includes a method of recombinantly producing a hybrid interferon fusion polypeptide using the expression vectors of the present invention. The expression vectors are introduced into suitable host cells. The host cells are then cultured under conditions that result in the expression of the ORF sequence.

10 In a further embodiment, the invention includes a method of recombinantly producing a hybrid interferon fusion polypeptide. In the method, the expression vector, containing sequences encoding a hybrid interferon fusion polypeptide open reading frame (ORF), is introduced into suitable host cells, where the vector is designed to express the ORF in the host cells. The transformed host cells are then cultured under conditions that result in the expression of the ORF

15 sequence. Numerous vectors and their corresponding hosts are useful in the practice of this method of the invention, including, lambda gt11 phage vector and *E. coli* cells. Other host cells include, yeast, mammalian cell, insect cell, tissue culture, plant cell culture, transgenic plants or bacterial expression systems.

The invention further includes a method of inhibiting tumor cell growth. In the method, the

20 tumor cells are contacted with a hybrid interferon fusion polypeptide at a concentration effective to inhibit growth of the tumor cells. The hybrid interferon fusion polypeptide may be a part of any acceptable pharmacological formulation. Tumor cells whose growth may be inhibited by a hybrid interferon fusion polypeptide include, but are not limited to, carcinoma cells, hematopoietic cancer cells, leukemia cells, lymphoma cells, and melanoma cells. In one embodiment, the tumor

25 cells are steroid-sensitive tumor cells, for example, mammary tumor cells.

In yet another aspect of the present invention, hybrid interferon fusion polypeptides are used in a method of inhibiting viral replication. In this method, cells infected with a virus are contacted with hybrid interferon fusion polypeptide at a concentration effective to inhibit viral replication within said cells. The hybrid interferon fusion polypeptide may be a part of any acceptable

30 pharmacological formulation. The replication of both RNA and DNA viruses may be inhibited by hybrid interferon fusion polypeptides. Exemplary RNA viruses include feline leukemia virus, ovine progressive pneumonia virus, ovine lentivirus, equine infectious anemia virus, bovine immunodeficiency virus, visna-maedi virus, caprine arthritis encephalitis virus, human immuno-

deficiency virus (HIV) or hepatitis c virus (HCV). An exemplary DNA virus is hepatitis B virus (HBV).

In still another aspect, the present invention includes a method of treating an autoimmune disease in a subject in need of such treatment. In one embodiment, the autoimmune disease is multiple sclerosis. The method includes administering, to the subject, a pharmaceutically effective amount of a hybrid interferon fusion polypeptide. The hybrid interferon (hybIFN) may be administered, for example, orally or via intravenous or intramuscular injection. Orally-administered hybIFN is preferably ingested by the subject.

Other embodiments of the invention include a methods of treating lupus erythematosus, type I diabetes, and rheumatoid arthritis in a subject in need of such treatment. The method includes administering, to the subject, a pharmaceutically effective amount of a hybrid interferon fusion polypeptide of the present invention.

These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Figures

Figures 1A, 1B, 1C, 1D and 1E present the nucleic acid coding sequence of a synthetic gene of OvIFNτ designed to include 19 unique restriction enzyme sites spaced evenly throughout the coding sequence.

Figure 2 shows the cloning strategy used for making a synthetic gene encoding OvIFNτ.

Figure 3 shows a comparison of the predicted protein sequences of a human interferon-τ gene and an ovine interferon-τ gene. Divergent amino acids are indicated by presentation of the alternative amino acid on the line below the nucleic acid sequences.

Figure 4 presents data demonstrating that both OvIFNτ and IFNα were able to drastically reduce growth of HL-60 cells.

Figure 5 presents data demonstrating that rHuIFNα is cytotoxic and OvIFNτ is not. In the figure, results of one of three replicate experiments are presented as mean % viability ± SD.

Figure 6 presents the sequences of polypeptides derived from the IFNτ sequence.

Figure 7 presents the complete nucleic acid and amino acid sequence of an OvIFNτ sequence.

Figure 8 presents data supporting the lack of cytotoxicity, relative to IFNα, when IFNτ is used to treat peripheral blood mononuclear cells.

Figure 9 shows the results of treatment of a human cutaneous T cell lymphoma line, HUT 78, with IFNτ.

Figure 10 shows the results of treatment of a human T cell lymphoma line, H9, with IFNτ.

Figure 11A presents data for the peptide inhibition, relative to FIV (feline immunodeficiency virus) replication, of polypeptides derived from OvIFNτ with whole OvIFNτ. Figure 11B presents data for the peptide inhibition, relative to HIV (human immunodeficiency virus) replication, of

5   polypeptides derived from OvIFNτ with whole OvIFNτ.

Figure 12 presents data demonstrating the inhibition of the antiviral activity of IFNτ by IFNτ-derived peptides.

Figure 13 presents data demonstrating the inhibition by IFNτ-derived peptides of OvIFNτ antiviral activity.

10   Figure 14 presents data demonstrating the inhibition by IFNτ-derived peptides of bovine IFNα antiviral activity.

Figure 15 presents data demonstrating the inhibition by IFNτ-derived peptides of human IFNα antiviral activity.

Figure 16 presents data evaluating the lack of inhibition by IFNτ-derived peptides of bovine

15   IFNγ antiviral activity.

Figure 17 presents data demonstrating the anti-IFNτ-derived peptide antisera inhibition of the antiviral activity of IFNτ.

Figure 18 presents data demonstrating the anti-IFNτ-derived peptide antisera inhibition of the binding of radiolabeled IFNτ to cells.

20   Figures 19A and 19B present an alignment of nucleic acid sequences encoding IFNτ polypeptides.

Figures 20A and 20B present an alignment of amino acid sequences of IFNτ polypeptides.

Figure 21 presents data comparing the cytotoxicity of IFNτ with IFNβ.

Figure 22A and 22B show cytotoxicity profiles of IFNαA (Fig. 22A) and IFNτ (Fig. 22B)

25   on MDBK cells.

Figures 23A and 23B show the effect of IFNτ on the cytotoxicity of IFNαA on MDBK cells.

Figures 24A and 24B show the binding of ^{125}I-IFNτ (Fig. 24A) and ^{125}I-IFNαA (Fig. 24B) to MDBK cells, as well as Scatchard plots of the binding data.

Figures 25A and 25B show competitive binding of IFNτ (Fig. 25A) and IFNαA (Fig. 25B)

30   to MDBK cells.

Figure 26 shows inhibition of binding of IFNτ and IFNαA to MDBK cells by antisera raised against the N terminus (solid bars) and C terminus (stippled bars) of IFNτ relative to pre-immune-treated controls (open bars).

Figures 27A and 27B show dose-response/occupancy curves for IFNτ (Fig. 27A) and IFNαA (Fig. 27B) on MDBK cells of percent maximal antiviral activity (○), cytotoxicity (□), and binding (◇).

Figure 28 shows a schematic of a hybrid interferon fusion polypeptide.

5 **Brief Description of the Sequences**

SEQ ID NO:1 is the nucleotide sequence of a synthetic gene encoding ovine interferon-τ (OvIFNτ). Also shown is the encoded amino acid sequence.

SEQ ID NO:2 is an amino acid sequence of a mature OvIFNτ protein.

SEQ ID NO:3 is a synthetic nucleotide sequence encoding a mature human interferon-τ

10 (HuIFNτ) protein.

SEQ ID NO:4 is an amino acid sequence for a mature HuIFNτ1 protein.

SEQ ID NO:5 is the amino acid sequence of fragment 1-37 of SEQ ID NO:2.

SEQ ID NO:6 is the amino acid sequence of fragment 34-64 of SEQ ID NO:2.

SEQ ID NO:7 is the amino acid sequence of fragment 62-92 of SEQ ID NO:2.

15 SEQ ID NO:8 is the amino acid sequence of fragment 90-122 of SEQ ID NO:2.

SEQ ID NO:9 is the amino acid sequence of fragment 119-150 of SEQ ID NO:2.

SEQ ID NO:10 is the amino acid sequence of fragment 139-172 of SEQ ID NO:2.

SEQ ID NO:11 is the nucleotide sequence of a natural HuIFNτ1 gene with a leader sequence.

SEQ ID NO:12 is the predicted amino acid coding sequence of the SEQ ID NO:11.

20 SEQ ID NO:13 is a 25-mer synthetic oligonucleotide according to the subject invention.

SEQ ID NO:14 is a 25-mer synthetic oligonucleotide according the subject invention.

SEQ ID NO:15 is the amino acid sequence of fragment 1-37 of SEQ ID NO:4.

SEQ ID NO:16 is the amino acid sequence of fragment 34-64 of SEQ ID NO:4.

SEQ ID NO:17 is the amino acid sequence of fragment 62-92 of SEQ ID NO:4.

25 SEQ ID NO:18 is the amino acid sequence of fragment 90-122 of SEQ ID NO:4.

SEQ ID NO:19 is the amino acid sequence of fragment 119-150 of SEQ ID NO:4.

SEQ ID NO:20 is the amino acid sequence of fragment 139-172 of SEQ ID NO:4.

SEQ ID NO:21 is the nucleotide sequence of cDNA HuIFNτ6.

SEQ ID NO:22 is the predicted amino acid sequence encoded by the sequence represented

30 as SEQ ID NO:21.

SEQ ID NO:23 is the nucleotide sequence of cDNA HuIFNτ7.

SEQ ID NO:24 is the predicted amino acid sequence encoded by the sequence represented as SEQ ID NO:23.

SEQ ID NO:25 is the nucleotide sequence of cDNA HuIFNτ4.

SEQ ID NO:26 is the predicted amino acid sequence encoded by the sequence represented as SEQ ID NO:25.

SEQ ID NO:27 is the nucleotide sequence of cDNA HuIFNτ5.

5   SEQ ID NO:28 is the predicted amino acid sequence encoded by the sequence represented as SEQ ID NO:27.

SEQ ID NO:29 is the nucleotide sequence of genomic DNA clone HuIFNτ2.

SEQ ID NO:30 is the predicted amino acid sequence encoded by the sequence represented as SEQ ID NO:29.

10   SEQ ID NO:31 is the nucleotide sequence, including leader sequence, of genomic DNA clone HuIFNτ3, a natural HuIFNτ gene.

SEQ ID NO:32 is the predicted amino acid sequence (including leader sequence) encoded by the sequence represented as SEQ ID NO:31.

SEQ ID NO:33 is the nucleotide sequence, excluding leader sequence, of genomic DNA clone

15 HuIFNτ3, a natural HuIFNτ gene.

SEQ ID NO:34 is the predicted amino acid sequence of a mature human IFNτ protein encoded by HuIFNτ3, encoded by the sequence represented as SEQ ID NO:33.

SEQ ID NO:35 is the amino acid sequence of fragment 1-37 of SEQ ID NO:33.

SEQ ID NO:36 is the amino acid sequence of fragment 34-64 of SEQ ID NO:33.

20   SEQ ID NO:37 is the amino acid sequence of fragment 62-92 of SEQ ID NO:33.

SEQ ID NO:38 is the amino acid sequence of fragment 90-122 of SEQ ID NO:33.

SEQ ID NO:39 is the amino acid sequence of fragment 119-150 of SEQ ID NO:33.

SEQ ID NO:40 is the amino acid sequence of fragment 139-172 of SEQ ID NO:33.

SEQ ID NO:41 is the amino acid sequence of fragment 1-23 of SEQ ID NO:32.

25   SEQ ID NO:42 is the amino acid sequence of fragment 1-23 of SEQ ID NO:11.

SEQ ID NO:43 is the nucleotide sequence, excluding leader sequence, of DNA clone HuIFNτ1.

SEQ ID NO:44 is the predicted amino acid sequence of a mature human IFNτ protein encoded by HuIFNτ1, encoded by the sequence represented as SEQ ID NO:43.

30   SEQ ID NO:45 is the predicted amino acid sequence of fragment 1-37 (mature sequence) from sequence A40068 (Bin 12, Accession #gi 108955), encoding bovine TP-1 (clone bTP509).

SEQ ID NO:46 is the predicted amino acid sequence of fragment 1-37 (mature sequence) from sequence BovTPH1Bcds1 (Bin 13, Accession #gi 163767), encoding bovine TP-1.

SEQ ID NO:47 is the predicted amino acid sequence of fragment 1-37 (mature sequence) from sequence BovTPH1Ccds1 (Bin 14, Accession #gi 163769), encoding bovine TP-1.

SEQ ID NO:48 is the predicted amino acid sequence of fragment 1-37 (mature sequence) from sequence A39505 (Bin 15, Accession #gi 163769), encoding bovine TP-1 (clone bTP4).

5   SEQ ID NO:49 is the predicted amino acid sequence of fragment 1-37 (mature sequence) from sequence OATP1P5cds1 (Bin 16, Accession #gi 1412), encoding ovine TPp5.

SEQ ID NO:50 is the predicted amino acid sequence of fragment 1-37 (mature sequence) from sequence OATP1P3cds1 (Bin 17, Accession #gi 1410), encoding ovine TPp3.

SEQ ID NO:51 is the predicted amino acid sequence of fragment 1-37 (mature sequence) 10   from sequence SHP010TPcds1 (Bin 18, Accession #gi 165821), encoding ovine TP-1.

SEQ ID NO:52 is the predicted amino acid sequence of fragment 1-37 (mature sequence) from sequence SHP02TPcds1 (Bin 19, Accession #gi 165823), encoding ovine TP-1.

SEQ ID NO:53 is the predicted amino acid sequence of fragment 1-37 (mature sequence) from sequence GOTCTPlcds1 (Bin 21, Accession #gi 164117), encoding Capra hircus IFN tau.

15   SEQ ID NO:54 is the nucleotide sequence of chimeric nucleic acid molecule derived from human DNA , with a 5'-end segment encoding amino acids 1-28 of human IFNtau (from SEQ ID NO:3) and a 3'-end segment encoding amino acids 29-167 of human IFNα (clone pIFN105; Genbank HUMIFNN Acc. M28585).

SEQ ID NO:55 is the predicted amino acid sequence from SEQ ID NO:54.

20   **Detailed Description of the Invention**

I.   Definitions.

Interferon-τ (IFNτ) refers to any one of a family of interferon proteins having greater than 70%, or preferably greater than about 80%, or more preferably greater than about 90% amino acid homology to either the sequence presented as (a) SEQ ID NO:2 or (b) SEQ ID NO:34. 25   Amino acid homology can be determined using, for example, the LALIGN program with default parameters. This program is found in the FASTA version 1.7 suite of sequence comparison programs (Pearson and Lipman 1988; Pearson, 1990; program available from William R. Pearson, Department of Biological Chemistry, Box 440, Jordan Hall, Charlottesville, VA). Typically, IFNτ has at least one characteristic from the following group of characteristics: (a) expressed during 30   embryonic/fetal stages by trophectoderm/placenta, (b) anti-luteolytic properties, (c) anti-viral properties, and (d) anti-cellular proliferation properties. IFNτ can be obtained from a number of sources including cows, sheep, ox, and humans.

An *interferon-τ polypeptide* is a polypeptide having between about 15 and 172 amino acids derived from an interferon-τ amino acid coding sequence, where said 15 to 172 amino acids are contiguous in native interferon-τ. Such 15-172 amino acid regions can also be assembled into polypeptides where two or more such interferon-τ regions are joined that are normally discon-

5   tinuous in the native protein.

A polynucleotide sequence or fragment is *"derived from"* another polynucleotide sequence or fragment when it contains the same sequence of nucleotides as are present in the sequence or fragment from which it is derived. For example, a bacterial plasmid contains an insert "derived from" a selected human gene if the sequence of the polynucleotides in the insert is the same as the

10   sequence of the polynucleotides in the selected human gene.

Similarly, a polypeptide sequence or fragment is *"derived from"* another polypeptide sequence or fragment when it contains the same sequence of amino acids as are present in the sequence or fragment from which it is derived.

Percent (%) identity, with respect to two amino acid sequences, refers to the % of residues

15   that are identical in the two sequences when the sequences are optimally aligned and no penalty is assigned to "gaps". In other words, if a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the % identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the "gap" of the first sequence). Optimal alignment is defined

20   as the alignment giving the highest % identity score. Such alignments can be preformed using the "GENEWORKS" program. Alternatively, alignments may be performed using the local alignment program LALIGN with a ktup of 1, default parameters and the default PAM.

Treating a disease refers to administering a therapeutic substance effective to reduce the symptoms of the disease and/or lessen the severity of the disease.

25   II. Isolation and Characterization of Interferon-τ.

A. Ovine and Bovine Interferon-τ.

1. Interferon-τ Coding Sequences.

Ovine interferon-τ (OvIFNτ) is a major conceptus secretory protein produced by the embryonic trophectoderm during the critical period of maternal recognition in sheep. One isolate

30   of mature OvIFNτ is 172 amino acids in length (SEQ ID NO:2). The cDNA coding sequence contains an additional 23 amino acids at the amino-terminal end of the mature protein (Imakawa, *et al.*, 1987). The coding sequence of this OvIFNτ isolate is presented as Figure 7.

15

Relative to other interferons, oIFNτ shares about 45 to 68% amino acid homology with Interferon-α and the greatest sequence similarity with the interferon-ωs (IFNωs) of about 68%.

For the isolation of OvIFNτ protein, conceptuses were collected from pregnant sheep and cultured *in vitro* in a modified Minimum Essential Medium as described previously (Godkin, *et al.*, 1982). Conceptuses were collected on various days of pregnancy with the first day of mating being described as Day 0. OvIFNτ was purified from conceptus culture medium essentially as described by Vallet, *et al.*, 1987 and Godkin, *et al.*, 1982.

The homogeneity of OvIFNτ was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Maniatis, *et al.*, 1982; Ausubel, *et al.*, 1988). Determination of protein concentration in purified OvIFNτ samples was performed using the bicinchoninic (BCA) assay (Pierce Chemical Co., Rockford, IL; Smith, P.K., *et al.*, 1985).

A homologous protein to OvIFNτ was isolated from cows (BoIFNτ; Helmer, *et al.*, 1987; Imakawa, *et al.*, 1989). OvIFNτ and BoIFNτ (i) have similar functions in maternal recognition of pregnancy, and (ii) share a high degree of amino acid and nucleotide sequence homology between mature proteins. The nucleic acid sequence homology between OvIFNτ and BoIFNτ is 76.3% for the 5' non-coding region, 89.7% for the coding region, and 91.9% for the 3' non-coding region. The amino acid sequence homology is 80.4%.

Example 1 describes the reproductive functions of OvIFNτ. OvIFNτ and recombinant human Interferon-α2 (rHuIFNα) were infused into uterine lumen of ewes at a variety of concentrations. The life span of the corpus luteum was assessed by examination of interestrous intervals, maintenance of progesterone secretion, and inhibition of prostaglandin secretion (Davis, *et al.*, 1992). Comparison of the data resulting from these examinations demonstrated a considerable lengthening of the interestrous interval when OvIFNτ is administered at 100μg/day and no meaningful effect when rHuIFNα is administered. These data support the conclusion that OvIFNτ significantly influences the biochemical events of the estrous cycle.

The antiviral properties of interferon-τ at various stages of the reproductive cycle were also examined (Example 2). Conceptus cultures were established using conceptus obtained from sheep at days 12 through 16 of the estrus cycle. Antiviral activity of supernatant from each conceptus culture was assessed. Culture supernatants had increasing antiviral activity associated with advancing development of the conceptus up to Day 16 post estrus.

2.   Recombinant Production of IFNτ

Recombinant OvIFNτ was produced using bacterial and yeast cells. The amino acid coding sequence for OvIFNτ was used to generate a corresponding DNA coding sequence with codon usage optimized for expression in *E. coli* (Example 3). The DNA coding sequence was

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synthetically constructed by sequential addition of oligonucleotides. Cloned oligonucleotides were fused into a single polynucleotide using the restriction digestions and ligations outlined in Figure 2. The polynucleotide coding sequence had the sequence presented as SEQ ID NO:1.

For expression of recombinant interferon polypeptides, such as synthetic OvIFNτ or the hybrid interferon fusion polypeptides of the present invention, the chimeric coding sequence can be placed in a number of bacterial expression vectors: for example, lambda gt11 (Promega, Madison WI); pGEX (Smith, D.B., et al., 1988); pGEMEX (Promega); and pBS (Stratagene, La Jolla CA) vectors. Other bacterial expression vectors containing suitable promoters, such as the T7 RNA polymerase promoter or the tac promoter, may also be used. Cloning of the OvIFNτ synthetic polynucleotide into a modified pIN III omp-A expression vector is described in Example 3. Production of the OvIFNτ protein was induced by the addition of IPTG. Soluble recombinant IFNτ was liberated from the cells by sonication or osmotic fractionation.

The protein can be further purified by standard methods, including size fractionation (column chromatography or preoperative gel electrophoresis) or affinity chromatography using, for example, anti-interferon antibodies (solid support available from Pharmacia, Piscataway NJ). Protein preparations can also be concentrated by, for example, filtration (Amicon, Danvers, Mass.).

The synthetic OvIFNτ gene was also cloned into the yeast cloning vector pBS24Ub (Example 4; Sabin, et al., 1989; Ecker, et al., 1989). Synthetic linkers were constructed to permit in-frame fusion of the OvIFNτ coding sequences with the ubiquitin coding sequences in the vector. The resulting junction allowed in vivo cleavage of the ubiquitin sequences from the OvIFNτ sequences.

The recombinant plasmid pBS24Ub-IFNτ was transformed into the yeast S. cerevisiae. Transformed yeast cells were cultured, lysed and the recombinant IFNτ (r-IFNτ) protein isolated from the cell lysates.

The amount of r-IFNτ was quantified by radioimmunoassay. Microsequencing of the purified r-IFNτ was carried out. The results demonstrated identity with native OvIFNτ through the first 15 amino acids. The results also confirmed that the ubiquitin/IFNτ fusion protein was correctly processed in vivo.

Recombinant IFNτ obtained by this method exhibited antiviral activity similar to the antiviral activity of IFNτ purified from conceptus-conditioned culture medium.

Other yeast vectors can be used in the practice of the present invention. They include 2 micron plasmid vectors (Ludwig, et al., 1993), yeast integrating plasmids (YIps; e.g., Shaw, et al., 1988), YEP vectors (Shen, et al., 1986), yeast centromere plasmids (YCps; e.g., Ernst, 1986), and the like. Preferably, the vectors include an expression cassette containing an effective

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yeast promoter, such as the MFα1 promoter (Ernst, 1986; Bayne, *et al.*, 1988), GADPH promoter (glyceraldehyde-3-phosphate-dehydrogenase; Wu, *et al.*, 1991), the galactose-inducible GAL10 promoter (Ludwig, *et al.*, 1993; Feher, *et al.*, 1989; Shen, *et al.*, 1986), or the methanol-regulated alcohol oxidase (AOX) promoter. The AOX promoter is particularly useful in *Pichia*

5   *pastoris* host cells (for example, the AOX promoter is used in pHIL and pPIC vectors included in the *Pichia* expression kit, available from Invitrogen, San Diego, CA).

The expression cassette may include additional elements to facilitate expression and purification of the recombinant protein, and/or to facilitate the insertion of the cassette into a vector or a yeast chromosome. For example, the cassette may include a signal sequence to direct secretion

10   of the protein. An exemplary signal sequence suitable for use in a variety of yeast expression vectors is the MFα1 pre-pro signal sequence (Bayne, *et al.*, 1988; Ludwig, *et al.*, 1993; Shaw, *et al.*, 1988). Other signal sequences may also be used. For example, the Pho1 signal sequence (Elliot, *et al.*, 1986) is particularly effective in *Pichia Pastoris* and *Schizosaccharomyces pombe* host cells.

15   Exemplary expression cassettes include (i) a cassette containing (5' to 3') the AOX promoter, the Pho1 signal sequence, and a DNA sequence encoding OvIFNτ, for expression in *P. pastoris* host cells, and (ii) a cassette containing (5' to 3') the MFα1 promoter, the MFα1 pre-pro signal sequence, and a DNA sequence encoding an interferon composition of the present invention, for expression in *S. cerevisiae* host cells.

20   Additional yeast vectors suitable for use with the present invention include, but are not limited to, other vectors with regulatable expression (Hitzeman, *et al.*, 1988; Rutter, *et al.*, 1988; Oeda, *et al.*, 1988). The yeast transformation host is typically *Saccharomyces cerevisiae*, however, as illustrated above, other yeast suitable for transformation can be used as well (*e.g.*, *Schizosaccharomyces pombe*, *Pichia pastoris* and the like).

25   The DNA encoding the an interferon polypeptide can be cloned into any number of commercially available vectors to generate expression of the polypeptide in the appropriate host system. These systems include the above described bacterial and yeast expression systems as well as the following: baculovirus expression (Reilly, *et al.*, 1992; Beames, *et al.*, 1991; Clontech, Palo Alto CA); plant cell expression, transgenic plant expression (*e.g.*, S.B. Gelvin and R.A.

30   Schilperoot, *Plant Molecular Biology*, 1988), and expression in mammalian cells (Clontech, Palo Alto CA; Gibco-BRL, Gaithersburg MD). These recombinant polypeptides can be expressed as fusion proteins or as native proteins. A number of features can be engineered into the expression vectors, such as leader sequences which promote the secretion of the expressed sequences into culture medium. The recombinantly produced polypeptides are typically isolated from lysed cells

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or culture media. Purification can be carried out by methods known in the art including salt fractionation, ion exchange chromatography, and affinity chromatography. Immunoaffinity chromatography can be employed, as described above, using antibodies generated based on the IFN polypeptides.

B. Human Interferon-τ

1. Identification and Cloning of Human Genomic Sequences Encoding an Interferon-τ Protein.

Human genomic DNA was screened for sequences homologous to interferon-τ (Example 5). Several sequences that hybridized with the OvIFNτ cDNA probe were identified. Several clones containing partial sequences of human interferon-τ were then isolated (Example 6). Two synthetic 25-mer oligonucleotides, corresponding to sequences from the OvIFNτ cDNA (Imakawa, et al., 1987; Whaley, et al., 1994) were synthesized. These primers were employed in amplification reactions using DNA derived from the following two cDNA libraries: human placenta and human cytotrophoblast cells isolated from term placenta. The resulting amplified DNA fragments were electrophoretically separated and a band containing human IFNτ amplification products was isolated. The amplification products were subcloned and the inserted amplification products sequenced using the dideoxy termination method.

Comparison of sequences from five of these clones revealed a high degree of sequence homology between the isolates, but the sequences were not identical. This result suggests the existence of multiple variants of human interferon-τ genes. Analysis of the nucleotide and protein sequences suggests that human interferon-τ genes may be classified on the basis of sequence homology into at least three groups. The groups are presented below.

Example 7 describes the isolation of several full-length human IFNτ genes. High molecular weight DNA was isolated from human peripheral blood mononuclear cells (PBMCs) and size-fractionated. Fractions were tested for the presence of IFNτ sequences using polymerase chain reaction: DNA molecules from fractions that tested amplification positive were used to generate a subgenomic library in λgt11.

This subgenomic library was plated and hybridized with an OvIFNτ cDNA probe (Example 7A). Approximately 20 clones were identified that hybridized to the probe. Plaques corresponding to the positive clones were passaged, DNA isolated and analyzed by amplification reactions using OvIFNτ primers. Of these twenty plaques, six plaques generated positive PCR signals. The phage from these six clones were purified and the inserts sequenced. One of the inserts from one of these six clones was used as a hybridization probe in the following screening.

Recombinant phage from the λgt11 subgenomic library were screened using the hybridization probe just described (Example 7B). Five clones giving positive hybridization signals were isolated

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and the inserts sequenced. The sequences from three of the clones overlapped, and the resulting consensus nucleic acid sequence (HuIFNτ1) is presented as SEQ ID NO.11 with the predicted protein coding sequence presented as SEQ ID NO:12. The predicted mature protein coding sequence is presented as SEQ ID NO:4. The sequences from the other two clones are presented

5   as SEQ ID NO:29 (HuIFNτ2) and SEQ ID NO:31 (HuIFNτ3). The predicted mature amino acid sequence from HuIFNτ2 is presented as SEQ ID NO:30. The predicted amino acid sequence from HuIFNτ3 is presented as SEQ ID NO:32, and the mature amino acid sequence as SEQ ID NO:34.

Comparison of the predicted protein sequences (Figure 3) of one of the human interferon-τ genes (SEQ ID NO:4) and the ovine interferon-τ gene demonstrates the levels of sequence

10   homology and divergence at the amino acid level.

An alignment of the nucleic acid sequences of the seven human interferon-τ nucleic acid sequences, described herein (Examples 6 and 7), with ovine interferon-τ is shown in Figures 19A and 19B. Sequences of OvIFNτ (oIFNτ), HuIFNτ1, HuIFNτ2, and HuIFNτ3 start at the upper left corner of Figure 19A with the initiation ATG codon and continue through the second page of

15   the figure. Sequences of HuIFNτ4, HuIFNτ5, HuIFNτ6 and HuIFNτ7 start approximately half-way down Figure 19A with the CAG codon at amino acid position 40 (to the right of the exclama-tion marks) and continue through the second page of the figure. The 5' and 3' ends of each of the clones for HuIFNτ4, HuIFNτ5, HuIFNτ6 and HuIFNτ7 are represented by exclamation marks.

The complete coding sequence of OvIFNτ is presented in the top row of each aligned set.

20   Nucleotides in the other sequences are indicated only at positions where they differ from those of OvIFNτ. Lower case letters represent nucleotide changes that do not result in amino acid changes, while upper case letters represent those changes that result in an amino acid substitution.

An alignment of the seven corresponding amino acid sequences, constructed in essentially the same manner as described above, is presented in Figures 20A and 20B. As above, the complete

25   amino acid sequence of OvIFNτ is presented in the top row, and amino acids of other sequences are indicated only at positions where they differ from the ovine sequence.

An examination of the alignments reveals that the seven sequences may be grouped into at least three groups. Group I contains HuIFNτ1 and HuIFNτ2, group II contains HuIFNτ3, HuIFNτ4 and HuIFNτ5, and group III contains HuIFNτ6 and HuIFNτ7. These groups may

30   represent families of interferon-τ genes having distinct cellular functions.

These groupings were established based on the following criteria. In mature proteins, Group I HuIFNτs have an asparagine (ASN) at amino acid position number 95 (numbers in reference to Figures 20A to 20B), a methionine (MET) at amino acid position number 104, and a leucine (LEU) at amino acid position number 120; Group II HuIFNτs have an aspartic acid (ASP) at

amino acid position number 95, a threonine (THR) at amino acid position number 104, and a methionine (MET) at amino acid position number 120; and Group III HuIFNτs have an arginine (ARG) at amino acid position number 72, a valine (VAL) at amino acid position number 120, and a serine (SER) at amino acid position number 122.

5   The nucleic acid and polypeptide human IFNτ sequences presented as SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34 can be used as the source for specific primers and probes to detect isolates of further human IFNτ

10   coding sequences and/or pseudogenes. Further, as described above, there may be more than one isoform of the IFNτ protein and more than one coding sequence per species. The specific nucleic acid probes used in the practice of the present invention and antibodies reactive with the IFNτ polypeptides may be useful to isolate unidentified variants of interferon-τ in mammals, according to the methods of the invention disclosed herein.

15   2.   Characterization of the Expression of Interferon-τ in Human Tissues.

Human placental cDNA libraries and an ovine cDNA library were analyzed by hybridization to the OvIFNτ cDNA probe (Example 8). This DNA hybridization analysis suggested that the IFNτ-signals from human cDNA libraries were approximately 1/100 of the signal obtained using the ovine cDNA library. OvIFNτ cDNAs constitute around 0.4% of the ovine cDNA library.

20   Accordingly, the abundance of human cDNAs responding to the OvIFNτ probe appears to be low, at least in the term placenta from which the cDNA libraries were generated.

The presence of HuIFNτ mRNA in human term placenta and amniocytes were also analyzed. The results suggest the presence of HuIFNτ mRNA in the feto-placental annex. The aminocytes also expressed the messages corresponding to OvIFNτ primers and human probe, suggesting that

25   the expression of IFNτ mRNA is not limited to the term placenta.

In addition, a RT-PCR analysis for the presence of HuIFNτ was applied to the total cellular RNA isolated from human adult lymphocytes: the results suggest that IFNτ mRNA exists in lymphocytes.

The expression of interferon-τ in human tissue was also examined using *in situ* hybridizatic

30   (Example 9). Sections from four healthy, different term and first trimester human placentas were examined. This analysis employed a cDNA probe derived from the OvIFNτ cDNA sequences (Example 9B). *In situ* hybridization was performed using an anti-sense RNA probe. In three separate experiments, specific hybridization was observed in all term and first trimester placental tissues.

First trimester placental villi (composed of an outer layer of syncytiotrophoblast, an under-
lying layer of cytotrophoblast, and a central stromal region with various types of mesenchymal
cells) displayed the highest transcript level of IFNτ in the cytotrophoblast cells. Less intense but
detectable levels were present in both the syncytiotrophoblast and stromal cells. A similar pattern
of transcript expression was demonstrated in the placental villi of term tissue but the level of signal
detection was low. First trimester extravillous trophoblasts displayed the highest amount of
message and stained positive when present in the maternal blood spaces within the decidua.

Howatson, *et al.*, (1988) noted IFNα production in the syncytiotrophoblast of chorionic villi
in both first trimester and term tissues. Also, Paulesu, *et al.* (1991) observed IFNα in extravillous
trophoblast as well as syncytiotrophoblasts, noting more intense and abundant reactivity in first
trimester placental tissue when compared to those taken at term. These investigators employed
antibodies raised against human IFNα subtypes, and none observed any IFNα in the villous
cytotrophoblasts.

The present results demonstrate that the human IFNτ gene is highly expressed in early
placental tissues by migrating extravillous trophoblasts, but is also expressed in villous
syncytiotrophoblasts, cytotrophoblasts, and various stromal cells. These results demonstrate the
detection of IFNτ transcripts in human pregnancy tissues, and IFNτ expression in the villous cyto-
trophoblasts as well as the extravillous trophoblast of first trimester placenta.

C. Antiviral Properties of Interferon-τ.

The antiviral activity of OvIFNτ has been evaluated against a number of viruses, including
both RNA and DNA viruses. The relative specific activity of OvIFNτ, purified to homogeneity,
was evaluated in antiviral assays (Example 10). OvIFNτ had a higher specific antiviral activity
than either rBoIFNα or rBoIFNγ (Example 10, Table 3).

One advantage of the present invention is that OvIFNτ has potent antiviral activity with
limited cytotoxic effects. Highly purified OvIFNτ was tested for anti-retroviral and cytotoxic
effects on peripheral blood lymphocytes exposed to feline AIDS and human AIDS retroviruses
(Bazer, *et al.*, 1989). The feline AIDS lentivirus produces a chronic AIDS-like syndrome in cats
and is a model for human AIDS (Pederson, *et al.*, 1987). Replication of either virus in peripheral
blood lymphocytes (PBL) was monitored by reverse transcriptase (RT) activity in culture
supernatants over time.

To determine IFNτ antiviral activity against FIV and HIV, RNA-dependent DNA polymerase
RT activity was assayed in FIV- and HIV-infected feline and human PBL cultures treated with
OvIFNτ (Example 11). Replication of FIV was reduced to about one-third of control values when
cells were cultured in the presence of OvIFNτ. Addition of OvIFNτ produced a rapid, dose-

dependent decrease in reverse transcriptase (RT) activity (Example 11, Table 4). While concentrations as low as 0.62 ng/ml of IFNτ inhibited viral replication, much higher concentrations (40 ng/ml) having greater effects on RT-activity were without toxic effects on the cells. The results suggest that replication of the feline immunodeficiency virus was reduced significantly

5 compared to control values when cells were cultured in the presence of OvIFNτ.

IFNτ appeared to exert no cytotoxic effect on the cells hosting the retrovirus. This was true even when IFNτ was present at 40 ng per ml of culture medium. This concentration of IFNτ is equivalent to about 8,000 antiviral units of alpha interferon--when OvIFNτ and HuIFNα are each assayed for their ability to protect Madin-Darby bovine kidney cells from lysis by vesicular

10 stomatitis virus (lysis assay as described by Pontzer, et al., 1988).

IFNτ was also tested for activity against HIV replication in human cells. Human peripheral lymphocytes, which had been infected with HIV were treated with varying concentrations of IFNτ (Example 12). Replication of HIV in peripheral blood lymphocytes was monitored by reverse transcriptase activity in culture supernatants over time. Over a range of concentrations of IFNτ

15 produced significant anti-HIV effects (Example 12, Table 5). A concentration of only 10 ng/ml resulted in over a 50% reduction in RT activity after only six days. A concentration of 500 ng/ml resulted in a 90% reduction in RT activity within 10 days. Further, there was no evidence of any cytotoxic effects attributable to the administration of IFNτ (Example 12, Table 5).

Further, the antiviral effects of IFNτ against HIV were evaluated by treating human PBMC

20 cells with various amounts of either recombinant IFNτ or recombinant human IFNα at the time of infection with HIV (Example 19). The data from these experiments (Example 19, Table 11) support the conclusion that, at similar concentrations, IFNα and IFNτ are effective in reducing the replication of HIV in human lymphocytes. However, treatment of cells with IFNα resulted in cytotoxicity, whereas no such cytotoxicity was observed with treatment using IFNτ, even when

25 IFNτ was used at much higher concentrations. No cytotoxicity was observed using IFNτ, even when IFNτ was used at 200 times the dosage of interferon-alpha II.

Both FIV and HIV reverse transcriptase themselves were unaffected by IFNτ in the absence of PBL. Therefore, the antiviral activity is not due to a direct effect on the viral RT.

Interferon-τ has also been shown to inhibit Hepatitis B Virus DNA replication in hepatocytes

30 (Example 19). A human cell line derived from liver cells transfected with Hepatitis B Virus (HBV) was used to test the antiviral effects of IFNτ. The cells were treated with both the IFNα and IFNτ over a range of concentrations. Both IFNα and IFNτ reduced DNA production by approximately two-fold compared to the no interferon control.

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To demonstrate that the effect of the interferons was specific to the infecting virus and not the result of an effect on general cell metabolism, the hepatocytes were examined for the effects of IFNα and IFNτ on hepatospecific mRNA production (Example 19). Two hepatocyte specific proteins, Apo E and Apo A1, were detected by hybridization analysis. There was no apparent 5 reduction of mRNA production for either hepatospecific mRNA at concentrations up to 40,000 units/ml of either IFNα or IFNτ. Further, no evidence for hepatotoxicity with IFNτ was seen in this assay.

The effects of recombinant ovine interferon tau (roIFNτ) on ovine lentivirus replication (OvLV) were also evaluated. *In vitro* effects were assayed by infecting goat synovial membrane 10 cells with serial dilutions of OvLV. The infected cells were treated daily with roIFNτ (0-2,500 antiviral units/ml [AVU/ml]) for 6 to 12 days, and virus replication and cytopathic effect (CPE; *e.g.*, as in Example 2) were evaluated.

Evaluation methods included viral growth curves, cell proliferation assay (*e.g.*, as in Examples 13, 14 or 15), syncytia formation assay (*e.g.*, as in Nagy, *et al.*, 1983; Dalgleish, *et* 15 *al.*, 1984), and quantitation of proviral DNA by PCR and reverse transcriptase assay (Mullis, 1987; Mullis, *et al.*, 1987). A reduction ($p < 0.001$) of OvLV titer and CPE (80-99%) was observed in the roIFNτ-treated cultures.

In vivo effects of roIFNτ were assayed by inoculating twenty-four newborn lambs with 5×10^6 TCID$_{50}$ of OvLV strain 85/34. Eleven of these lambs were treated with 10^5-10^6 AVU/Kg of 20 roIFNτ once a day for 30 days post-inoculation (PI) and twice a week thereafter. Thirteen lambs were used as controls. Virus titers in blood, as determined by an end point dilution method, peaked at 4-6 weeks PI in both groups. OvLV titers in roIFNτ-treated lambs were reduced relative to control animals. The largest reduction, a 90% decrease in OvLV titer in treated animals relative to control animals ($p < 0.01$), was obtained 4 weeks PI.

25 The OvLV studies described above indicate that recombinant ovIFNτ can significantly reduce OvLV replication, and suggest that IFNτ may be used to control clinical diseases caused by lenti-virus infections. Taken together with the other antiviral data, these results suggest that IFNτ is an effective antiviral agent against a wide variety of viruses, including both RNA and DNA viruses.

30 Interferon compositions of the present invention may be useful in veterinary applications including, but not limited to, the treatment of the following viral diseases: feline leukemia virus, ovine progressive pneumonia virus, ovine lentivirus, equine infectious anemia virus, bovine immunodeficiency virus, visna-maedi virus, and caprine arthritis encephalitis.

Human-derived interferon compositions may be used for the treatment of, for example, the following viral diseases: human immunodeficiency virus (HIV), hepatitis c virus (HCV) and hepatitis B virus (HBV).

D. Antiproliferative Properties of IFNτ.

The effects of IFNτ on cellular growth have also been examined. In one analysis, anti-cellular growth activity was examined using a colony inhibition assay (Example 13). Human amnion (WISH) or MDBK cells were plated at low cell densities to form colonies originating from single cells. Dilutions of interferons were added to triplicate wells and the plates were incubated to allow colony formation. IFNτ inhibited both colony size and number in these assays. IFNτ was more effective at inhibiting cell proliferation of the human cell line (WISH) than human IFNα. The antiproliferative activity of IFNτ was dose-dependent. High concentrations of IFNτ stopped proliferation, while cell viability was not impaired.

Based on cell cycle analysis, using flow cytometry, IFNτ appears to inhibit progress of cells through S phase. These results demonstrate the antiproliferative effect of IFNτ, and underscore its low cytotoxicity.

The antiproliferative effects of IFNτ were also studied for rat and bovine cell lines (Example 14). The rate of ^{3}H-thymidine incorporation was used to assess the rate of cellular proliferation. The data obtained demonstrate that IFNτ drastically reduced the rate of cellular proliferation (Example 14, Table 7) for each tested cell line.

The antiproliferative activity and lack of toxicity of IFNτ was further examined using a series of human tumor cell lines (Example 15). A variety of human tumor cell lines were selected from the standard lines used in NIH screening procedure for antineoplastic agents (Pontzer, et al., 1991). At least one cell line from each major neoplastic category was examined.

The following cell lines were obtained from American Type Culture Collection (12301 Parklawn Dr., Rockville MD 20852):

NCI-H460	human lung large cell carcinoma;
DLD-1	human colon adenocarcinoma;
SK-MEL-28	human malignant melanoma;
A ⁷N	human renal adenocarcinoma;
H)	human promyelocytic leukemia;
H9	human T cell lymphoma;
HUT 78	human cutaneous T cell lymphoma; and
MCF7	human breast adenocarcinoma.

As above, the antiproliferative activity was evaluated by measuring the rate of ^{3}H-thymidine incorporation into cells which have been treated with IFNτ. Significant differences between

treatments were assessed by an analysis of variance followed by Scheffe's F-test. Cell cycle analysis was performed by flow cytometry.

Examination of IFNτ inhibition of MCF7 (breast adenocarcinoma) proliferation demonstrated that IFNτ reduced MCF7 proliferation in a dose-dependent manner. A 50% reduction in ^{3}H-
5   thymidine was observed with 10,000 units/ml of IFNτ (Example 15, Table 8). This cell line had previously been found to be unresponsive to anti-estrogen treatment.

A comparison of the antiproliferative effects of IFNτ and IFNα was conducted using HL-60 (human promyelocytic leukemia) cells. Results with the promyelocytic leukemia HL-60 are typical of those obtained comparing IFNτ with human IFNα (Example 15). Concentrations as low as 100
10   units/ml of both IFNs produced significant (> 60%) growth reduction. Increasing amounts of IFNs further decreased tumor cell proliferation (Figure 4). High doses of HuIFNα, but not OvIFNτ, were cytotoxic (Figure 5). Cell viability was reduced by approximately 80% by IFNα. By contrast, nearly 100% of the IFNτ-treated cells remained viable when IFNτ was applied at 10,000 units/ml. Thus, while both interferons inhibit proliferation, only IFNτ is without
15   cytotoxicity. This lack of toxicity provides an advantage of IFNτ for use in *vivo* therapies.

The human cutaneous T cell lymphoma, HUT 78, responded similarly to HL-60 when treated with IFNτ (Example 15, Figure 9). Both OvIFNτ and rHuIFNα reduce HUT 78 cell growth, but IFNα demonstrated adverse effects on cell viability.

The T cell lymphoma H9 was less sensitive to the antiproliferative effects of IFNα than the
20   tumor cell lines described above. While IFNα was not toxic to the H9 cells, it failed to inhibit cell division significantly at any of the concentrations examined (Example 15, Figure 10). In contrast, IFNτ was observed to reduce H9 growth by approximately 60%. Thus, only OvIFNτ is an effective growth inhibitor of this T cell lymphoma.

In three additional tumor cell lines (NCI-H460, DLD-1 and SK-MEL-28) IFNτ and IFNα
25   were equally efficacious antitumor agents. In the melanoma, SK-MEL-28, inhibition of proliferation by IFNα was accomplished by a 13% drop in viability, while IFNτ was not cytotoxic. In the majority of tumors examined, IFNτ is equal or preferable to IFNα as an antineoplastic agent against human tumors.

IFNτ exhibits antiproliferative activity against human tumor cells without toxicity and is as
30   potent or more potent than human IFNα. Clinical trials of the IFNα2s have shown them to be effective antitumor agents (Dianzani, 1992; Krown, 1987). One advantage of IFNτ as a therapeutic is the elimination of toxic effects seen with high doses IFNαs.

An additional application of the IFNτ is against tumors like Kaposi's sarcoma (associated with HIV infection) where the antineoplastic effects of IFNτ are coupled with IFNτ ability to inhibit retroviral growth.

The *in vivo* efficacy of interferon-τ treatment was examined in a mouse system (Example 16).

5   B16-F10 is a syngeneic mouse transplantable tumor selected because of its high incidence of pulmonary metastases (Poste, *et al.*, 1981). Interferon treatment was initiated 3 days after the introduction of the tumor cells. The *in vivo* administration of IFNτ dramatically reduced B16-F10 pulmonary tumors. Thus, IFNτ appears to be an efficacious antineoplastic agent *in vivo* as well as *in vitro*.

10   These results suggest that interferon compositions of the present invention may be used in methods to inhibit or reduce tumor cell growth, including, but are not limited to, the following types of tumor cells: human carcinoma cells, hematopoietic cancer cells, human leukemia cells, human lymphoma cells, human melanoma cells and steroid-sensitive tumor cells (for example, mammary tumor cells).

15   E.   Type I IFNs as Treatment for Autoimmune Disorders.

Compositions and methods of the present invention may be used to therapeutically treat and thereby alleviate a variety of immune system-related disorders characterized by hyper- or hypo-active immune system function. Such disorders include hyperallergenicity and autoimmune disorders, such as multiple sclerosis, type I (insulin dependent) diabetes mellitus, lupus

20   erythematosus, amyotrophic lateral sclerosis, Crohn's disease, rheumatoid arthritis, stomatitis, asthma, allergies, psoriasis and the like.

F.   Effectiveness of Orally-Administered IFNτ

Experiments performed in support of the present invention demonstrate that orally-adminis-tered IFNτ polypeptide compositions are comparable in efficacy to injected IFNτ compositions

25   with respect to the treatment of diseases or disease conditions which benefit from treatment with IFNτ, such as autoimmune diseases (*e.g.*, multiple sclerosis).

Not only was orally-administered IFNτ effective at treating a disease benefiting from IFNτ treatment (EAE), but the oral route of administration resulted in unexpected advantages relative to treatment with injected IFNτ compositions. For example, orally-administered IFNτ resulted in

30   a significantly lower level of anti-IFNτ antibodies in the serum of treated individuals. This is beneficial because the orally-administered IFNτ is therefore less likely to be rendered ineffective by a host immune response (*i.e.*, desensitization to the treatment and/or dose level is significantly decreased), and the individual receiving the treatment is less likely to suffer adverse side effects as a result of such an immune response.

G. Cytotoxicity of Interferons.

One advantage of IFNτ over other interferons, such as IFNα, is that treatment of a subject with therapeutic doses of IFNτ does not appear to be associated with cytotoxicity. In particular, IFN-τ appears to be non-toxic at concentrations at which IFN-β induces toxicity. This is demonstrated by experiments in which L929 cells were treated with various concentrations of either oIFNτ or MuIFN-β (Lee Biomolecular, San Diego, CA), ranging from 6000 U/ml to 200,000 U/ml (Example 19E).

oIFNτ, MuIFN-β or medium (control) were added at time zero and the cells were incubated for 72 hours. The results of the experiments are presented in Figure 21. The percent of live cells (relative to control) is indicated along the y-axis (± standard error). One hundred percent is equal to the viability of L929 cells treated with medium alone. The results indicate that oIFNτ is essentially non-toxic at concentrations up to 100,000 U/ml, and is significantly less toxic than MuIFN-β over the entire therapeutic range of the compounds.

It has been previously demonstrated that *in vivo* treatment with both of the type I IFNs, IFNβ and IFNα in humans and animals causes toxicity manifested as a number of side effects including fever, lethargy, tachycardia, weight loss, and leukopenia (Degre, 1974; Fent and Zbinden, 1987). The effect of *in vivo* treatment with IFNτ, IFNβ and IFNα (10^5 U/injection) on total white blood cell (WBC), total lymphocyte counts and weight measurements in NZW mice (Table 13) was examined as described in Example 19F. No significant difference between IFNτ treated and untreated mice was observed for WBC, lymphocyte counts or weight change.

In comparison, IFNβ treated mice exhibited a 31.7% depression in lymphocyte counts 12 hours after injection. Further, depression of lymphocyte counts continued 24 hours after IFNβ injection. IFNα treated mice exhibited a 55.8% lymphocyte depression and significant weight loss 12 hours after injection. Additional experiments performed in support of the present invention demonstrated that IFNτ does not suppress bone marrow at high doses. Thus, IFNτ appears to lack toxicity *in vivo* unlike IFNβ and IFNα as evidenced by studies of peripheral blood and weight measurements. As is described below, experiments performed in support of the present invention indicate that the reduced toxicity of IFNτ with respect to IFNβ and IFNα, as summarized above, may be due to sequences in present in the N-terminal 37 amino acids of IFNτ, and that substitution of these sequences for the corresponding sequences in non-tau type I interferons, such as IFNβs and/or IFNαs, may confer such reduced cytotoxicity on the resulting hybrid interferon fusion polypeptides.

III. Interferon-τ Polypeptide Fragments and Differential Recognition of the Type I Interferon Receptor by IFN-τ and IFN-α.

A. IFN-τ Polypeptide Fragments.

The variety of IFNτ activities, its potency and lack of cytotoxicity, as taught by the present

5   specification, suggest the importance of structure/function analysis for this novel interferon. The structural basis for OvIFNτ function has been examined using six overlapping synthetic peptides corresponding to the entire OvIFNτ sequence (Figure 6). The corresponding polypeptides derived from the ovine IFNτ sequence are presented as SEQ ID NO:5 to SEQ ID NO:10. Three peptides representing amino acids 1-37, 62-92 and 139-172 have been shown to inhibit IFNτ antiviral

10   activity (Example 17). The peptides were effective competitors at concentrations of 300 μM and above.

The synthetic polypeptide representing the C-terminal region of ovIFNτ, OvIFNτ (139-172), and the internal peptide OvIFNτ (62-92), inhibited IFNτ and rBoIFNα₁₁ antiviral activity to the same extent, while the N-terminal peptide OvIFNτ (1-37) was more effective in inhibiting OvIFNτ

15   antiviral activity. Dose-response data indicated that IFNτ (62-92) and IFNτ (139-172) inhibited IFNτ antiviral activity to similar extents. The same peptides that blocked IFNτ antiviral activity also blocked the antiviral activity of recombinant bovine IFNα (rBoIFNα); recombinant bovine IFNγ was unaffected by the peptides. These two IFNτ peptides may represent common receptor binding regions for IFNτ and various IFNαs.

20   The two synthetic peptides OvIFNτ(1-37) and OvIFNτ(139-172) also blocked OvIFNτ anti-FIV and anti-HIV activity (Example 17; Figures 11A and 11B). While both peptides blocked FIV RT activity, only the C-terminal peptide, OvIFNτ(139-172), appeared to be an efficient inhibitor of vesicular stomatitis virus activity on the feline cell line, Fc9.

Polyclonal anti-peptide antisera against the IFNτ peptides yielded similar results as the

25   polypeptide inhibition studies, described above. Antibodies directed against the same three regions (OvIFNτ (1-37), IFNτ (62-92) and IFNτ (139-172)) blocked OvIFNτ function, confirming the importance of these three domains in antiviral activity (Example 17). These peptides, although apparently binding to the interferon receptor, did not in and of themselves elicit interferon-like effects in the cells.

30   The antiproliferative activity of IFNτ (Example 17, Table 11) involved a further region of the molecule, since IFNτ(119-150) was the most effective inhibitor of OvIFNτ-induced reduction of cell proliferation. This results suggests that the region of the molecule primarily responsible for inhibition of cell growth is the IFNτ(119-150) region. This region of the IFNτ molecule may be useful alone or fused to other proteins (such as serum albumin, an antibody or an interferon

35   alpha polypeptide) as an antineoplastic agent. A conjugated protein between an N-terminal peptide

derived from human interferon-α and serum albumin was shown to have anticellular proliferation activity (Ruegg. et al., 1990).

Finally, binding of ¹²⁵I-OvIFNτ to its receptor on MDBK cells could be blocked by antisera to 4 of the 6 peptides: the 4 polypeptides representing amino acids 1-37, 62-92, 119-150 and 139-172 of OvIFNτ. This reflects the multiple binding domains as well as the functional significance of these regions. Since different regions of IFNτ are involved in elicitation of different functions, modification of selected amino acids could potentially result in IFNτ-like interferons with selective biological activity.

Polypeptide fragments of human IFNτ proteins, having similar properties to the OvIFNτ polypeptides just described, are proposed based on the data presented above for OvIFNτ polypeptide fragments combined with the HuIFNτ sequence information disclosed herein. Such human-sequence derived polypeptides include, but are not limited to, the following: SEQ ID NO:15 to SEQ ID NO:20, and SEQ ID NO:35 to SEQ ID NO:40.

B.   Effects and Interactions of IFN-τ and IFN-α at a Type-I Interferon Receptor.

Consistent with the peptide antagonist studies described above and in Example 17, the experiments described in Example 18 show that high concentrations (up to a 10-fold excess) of OvIFNτ failed to compete for receptor and block the toxic effects of human IFNαA on MDBK cells. A comparison of the relative antiviral, cytotoxic, receptor binding, and receptor competition properties of OvIFNτ and human IFNαA provides insight at the level of ligand-receptor interactions. IFNτ and IFNαA possess similar specific antiviral activities, as previously shown (Pontzer, et al. 1988). However, as shown in Example 18, IFNαA has an ≈ 10-fold lower K_d for receptor than does IFNτ and, hence, a higher binding affinity for receptor. Moreover, IFNαA is severalfold more effective than IFNτ in binding competition assays using either ¹²⁵I-IFNτ or ¹²⁵I-IFNαA (Figs. 24A, 24B). Since the numbers of binding sites per cell for IFNτ and IFNαA are very similar, and IFNτ competes with IFNαA binding, it appears that IFNαA and IFNτ recognize the same receptor complex.

A comparison of dose-response/occupancy curves for the cytotoxicities and antiviral activities (Figs. 27A and 27B) shows that cytotoxicity is associated with maximal receptor occupancy and hence binding affinities; IFNαA has a greater binding affinity and thus possesses substantially greater toxicity. On the other hand, antiviral activity is maximal at concentrations that result in only a very small fractional occupancy of receptors and is not represented by equilibrium binding data.

Experiments performed in support of the present invention have also shown that ovine IFNτ, like IFNαA, can induce the very rapid phosphorylation of the type I receptor-associated kinase

Tyk2 and the transcription factors Statlα and Stat2. Given the time scales of the stimulation by IFNτ and IFNαA, it appears that only a small fraction of the receptors need to be occupied in order to induce the phosphorylation of Tyk2, Statlα, and Stat2. Taken together, these data suggest that phosphorylation of these signal transduction proteins may not be sufficient to induce the
5 cellular toxicity associated with "other" type I IFNs (*i.e.*, type I IFNs other than IFN-tau).

The higher binding affinity of IFNαA for receptor and the differential competition properties of IFNτ and IFNαA also suggest that the two IFNs recognize the receptor differently. Experiments performed in support of the present invention using synthetic peptide antagonists, including experiments described in Example 17, showed that the C-terminal peptide 1FNτ--
10 (139-172) (SEQ ID NO:10) was competitive against the activities of both IFNαA and IFNτ, whereas the N-terminal peptide IFNτ-(1-37) (SEQ ID NO:5) was effective at a 5- to 10-fold higher concentration only against IFNτ activity. Experiments using antisera raised against IFNτ-- (139-172) and IFNτ-(1-37) have shown that antiserum to IFNτ-(1-37) blocks only the binding of IFNτ, whereas antiserum to IFNτ(139-172) blocks the binding of both IFNα and IFNτ. These
15 data suggest that the N-terminal portions of IFNτ and IFNαA represent significant determinants of high-affinity binding and that differences in high-affinity equilibrium binding between IFNαA and IFNτ are due to differences in receptor interactions at the N termini of those molecules. Accordingly, the N termini of these molecules also appear to be significant determinants of the cytotoxic effects of the IFNs.

20   C.   Hybrid Interferon Fusion Proteins.

The above data taken together suggest that the C-terminal regions of type I interferons bind to a common site on the type I interferon receptor (*i.e.*, at a site that affects the receptor activation properties of both IFNα and IFNτ), while the N-terminal region may be involved in the elicitation of unique functions (*i.e.*, binds at a site that affects the receptor activation properties of only
25 IFNτ). In particular, the data suggest that the N-terminal region is responsible for the decreased cytotoxicity of IFNτ relative to other type I interferons, *e.g.*, IFNα.

The present invention employs observations regarding decreased toxicity conferred by the N-terminal region of IFNτ in support of chimeric DNA constructs used to produce hybrid interferon fusion proteins having an N-terminal portion derived from IFNτ and a C-terminal portion derived
30 from a non-tau interferon type I polypeptide whose effectiveness as a therapeutic is diminished by its relatively high cytotoxicity. Examples of such non-tau interferon type I polypeptides include IFNβ and the various isoforms of IFNα.

With reference to Fig. 28, such a hybrid interferon fusion protein or polypeptide **40**, encoded by a chimeric nucleic acid molecule, has an N-terminus **42** and a C-terminus **44**. The fusion

protein is made up of a first (N-terminal) segment 46 and a second (C-terminal) segment 48. The N-terminal segment contains the N-terminal amino acid sequence of an interferon-tau polypeptide encoded by a 5' end segment of the chimeric nucleic acid molecule. The C-terminal segment contains the C-terminal amino acid sequence of a non-tau interferon type I polypeptide and amino

5   acid sequence of an interferon-tau polypeptide encoded by a 3' end segment of the chimeric nucleic acid molecule. The two segments are joined or spliced at a junction point 50 which is in a region (junction region) corresponding to the portion of a mature interferon polypeptide between about amino acid residues 8 and 37. Note that the mature IFNτ polypeptide typically begins with a cysteine at amino acid 24 of the complete sequence (which includes the leader sequence and begins

10   with a methionine).

The junction region is contained in the 37 amino acid N-terminal peptide (SEQ ID NO:5) employed in the experiments detailed above. An alignment of the mature amimo acid sequences of several IFNτ, IFNα and IFNβ clones between amino acids 1 and 37 revealed that the greatest degree of divergence among the sequences occurs close to the N-terminus. In particular, the

15   highest degree of divergence between sequences occurs between amino acids 1 and 16, with an intermediate degree of divergence between amino acids 17 and 28. The region between amino acids 29 and 37 is relatively well-conserved among the different type-I interferons, and is thought to be involved in type-I interferon receptor binding interactions (Fish, 1992)

The optimal junction (*i.e.*, the amino acid residue position upstream of which (towards the

20   N-terminal or 5' end) the sequence corresponds to interferon tau, and downstream of which (towards the C-terminal or 3' end) it corresponds to another interferon, *e.g.*, IFNα or IFNβ can be identified, *e.g.*, by the methods described herein, using peptides or DNA sequences encoding peptides corresponding to longer and shorter regions within IFNτ (1-37), in combination with the functional assays described herein (such as, antiviral, antiproliferative and cytotoxicity assays).

25   It is contemplated that, for example, that a hybrid or chimeric interferon of the present invention containing amino acids 1-28 of interferon tau and the remaining amino acids from a non-tau type I interferon possesses the low toxicity associated with interferon tau along with the biological activity associated normally ascribed to such a type I interferon. For example, an IFNτ/IFNα hybrid may, for example, reduce the toxicity of IFNα but not interfere with IFNα antiviral

30   properties.

As stated above, interferon fusion proteins of the present invention have an amino acid sequence where the first about 8 to 37 amino acids of the mature interferon protein have a sequence of an IFNτ molecule, and the remaining amino acids have a sequence of a non-tau interferon type I polypeptide. Exemplary sequences from which the first 8-37 amino acids of the

interferon fusion protein may be selected include the sequences presented herein as SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:35, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53.

The remaining sequence (*i.e.*, the amino acid sequence of the "second", C-terminal segment,
5 which is encoded by 3' end segment of the chimeric nucleic acid molecule) may be selected from any suitable non-tau interferon type I polypeptide, such as interferon alpha (e.g., alpha-1 or alpha-2), interferon beta, interferon omega, a hybrid interferon or a consensus interferon. Sequences for non-tau type I interferons are known in the art. For example, exemplary sequences of hybrid interferons are provided by Gunther, *et al.*, 1990, Leibowitz, *et al.*, 1990, Goeddel, 1983, 1984,
10 1987, and Creasey, *et al.*, 1986, 1988). Exemplary sequences of consensus interferons are provided by Stabinsky, 1990. Exemplary interferon alpha sequences are presented in Capon, 1983, Dworkin-Rastl, 1989, Sato, 1988 and Sloma, 1988. Additional interferon alpha and beta sequences are provided in Fish, 1992. Suitable sequences may also be obtained from GenBank or other public sequence depository.

15 Determination of the non-tau type I interferon amino acid residue position with which the 3'-end or C-terminal segment begins is accomplished by optimally-aligning the parent sequences and engineering a junction such that the sequence of the resulting chimeric interferon molecule is aligned perfectly with (i) the interferon tau parent sequence in the 5' end or N-terminal segment, and (ii) the non-tau type I interferon parent sequence in the 3' end or C-terminal segment. The
20 parent sequences, of course, are the interferon sequences from which the 5' end or N-terminal segment and the 3' end or C-terminal segment are derived.

The residue position in the non-tau type I interferon with which the 3'-end or C-terminal segment begins is typically the number following the last amino acid residue of the 5'-end or N-terminal IFN-tau segment. For example, in a hybrid interferon fusion protein where the first ten
25 amino acids have the sequence of the first ten amino acids of SEQ ID NO:5, the remaining amino acids may have the sequence of mature interferon alpha (*e.g.*, IFN-αCon$_1$ as described in Fish, 1992) minus the first ten amino acids of the interferon alpha.

Preferred embodiments of the present invention are fusion proteins where the sequences of both the N-terminal and the C-terminal segments are derived from human interferon sequences.
30 For example, constructs where the first, *e.g.*, 28 amino acids correspond to the first 28 amino acids of SEQ ID NO:15 or SEQ ID NO:35, and the remaining sequence corresponds to that of a human interferon alpha or beta. Human interferon tau sequences are presented, *e.g.*, in Bazer, *et al.*, 1994. Human interferon alpha and beta sequences may be obtained from GenBank.

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It will be appreciated that although the interferon fusion proteins described are "mature" proteins, that is, they begin with residue 24 of the complete interferon sequence, the invention also includes fusion proteins and chimeric nucleic acid molecules encoding fusion proteins that contain the leader sequence, *i.e.*, that begin with the initiation methionine. The leader sequence in such
5   interferon fusion proteins may be derived from either a tau or non-tau type I interferon.

Further, it will be understood that the sequences of both the first and second fragments may be "consensus" sequences. In other words, the sequence of the 5' segment, might not correspond to a "natural" IFNτ, but to a consensus sequence obtained by comparing aligned sequences from several different IFNτs. Similarly, the sequence of the 3' segment, might not correspond to a
10   "natural" non-tau type I IFN, but to a consensus sequence obtained by comparing aligned sequences from several different non-tau type I IFNs.

Alternatively, the sequence of either fragment may correspond to an "internally-consistent" sequence, *i.e.*, to a sequence where each position in the sequence contains a residue that is found in at least one naturally-occurring isoform of either an IFNτ (for the N-terminal segment) or a
15   non-tau type I IFN (for the C-terminal segment) at that position, but where the final sequence corresponds to neither any naturally-occurring isoform nor to any consensus sequence. For example, if two isoforms, each 3 amino acids in length, have the sequences "C R S" and "C K G", an internally-consistent sequence is "C R G".

Furthermore, it will be appreciated that the present invention also includes more complex
20   chimeras, *e.g.*, chimeras containing more than one discrete region derived from IFNτ and/or more than one region from another suitable interferon. Such chimeras may arise, for example, in cases where the non-tau type I interferon comprising the second (C-terminal) segment is itself a hybrid interferon formed of, *e.g.*, an alpha interferon and a beta interferon (Creasey, *et al.*, 1986, 1988), an alpha-1 and an alpha-2 interferon (Leibowitz, *et al.*, 1990) or an alpha interferon and an omega
25   interferon (Gunther, *et al.*, 1990).

As pointed out above, a considerable advantage contemplated for hybrid interferon fusion protein compositions of the present invention is reduced toxicity of the compositions relative to native non-tau type I interferons that have, for example, been approved as therapeutics. The hybrid compositions may have the same biological activity as the approved non-tau type I IFNs
30   with the decreased cytotoxicity of IFNtau.

Chimeric nucleic acid molecules may be produced synthetically or with standard molecular protocols and manipulations (Ausubel, *et al.*, 1988; Sambrook, *et al.*, 1989), as exemplified herein. DNA sequences encoding the parent polypeptides (the two polypeptides from whose sequences the two segments forming the hybrid protein are derived) are cloned adjacent one

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another in an expression vector using standard recombinant methods (e.g., by engineering restriction sites which do not alter the translated amino acid sequence into the DNA sequences, digesting the plasmids, and cloning the appropriate fragments into the selected expression vector). Examples of suitable expression vectors are described above.

5   Recombinant hybrid interferon fusion polypeptides are then produced from such expression vectors as described above, purified and employed for the treatment of diseases and/or conditions which benefit from interferon treatment.

IV. Protein Modeling and Protein Modifications.

The data in the sections above demonstrate the identification of synthetic peptides having four

10   discontinuous sites on the OvIFNτ protein that are involved in receptor interaction and biological activity. In order to elucidate the structural relationship of these regions, modeling of the three dimensional structure of IFNτ was undertaken. A three dimensional model would be useful in interpretation of existing data and the design of future structure/function studies.

A. Molecular Protein Modeling

15   Combining circular dichroism (CD) data of both the full length recombinant OvIFNτ and IFNβ (a protein of known three dimensional structure (Senda, et al., 1992), a model of OvIFNτ was constructed. The most striking feature of this model is that IFNτ falls into a class of proteins with a four-helix bundle motif. The CD spectra of IFNτ was taken on an AVIV 60 S spectropolarimeter. Two different methods were employed for secondary structure estimations, the

20   algorithm of Perczel, et al., (1991) and variable selection by W.C. Johnson, Jr. (1992).

Secondary structure estimations of the spectra indicate 70-75% alpha helix (characterized by minima at 222 and 208 nm and maximum at 190 nm). The variable selection algorithm estimates the remainder of the molecule to be 20% beta sheet and 10% turn. The Chang method estimates the remainder to be 30% random coil. Alignment of IFNτ and IFNβ sequences revealed

25   homology between the two molecules, specifically in the regions of known helical structure in IFNβ. Sequence analysis of IFNτ also showed that proposed helical regions possess an apolar periodicity indicative of a four-helix bundle motif.

The final modeling step was to apply the IFNβ x-ray crystallographic coordinates of the IFNβ carbon backbone to the IFNτ sequence. The functionally active domains of IFNτ, identified

30   above, were localized to one side of the molecule and found to be in close spatial proximity. This is consistent with multiple binding sites on IFNτ interacting simultaneously with the type I IFN receptor.

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The three dimensional modeling data coupled with the function data described above, provides the information necessary to introduce sequence variations into specific regions of IFNτ to enhance selected functions (e.g., antiviral or anticellular proliferation) or to substitute a region(s) of selected function into other interferon molecules (e.g., antiviral, antineoplastic, or reduced cytotoxicity).

B.   Recombinant and Synthetic Manipulations

The construction of a synthetic gene for OvIFNτ is described in Example 3. Briefly, an amino acid sequence of ovIFNτ was back-translated from an ovIFNτ cDNA (Imakawa, et al., 1987) using optimal codon usage for E. coli. The sequence was edited to include 20, unique, restriction sites spaced throughout the length of the construct. This 540 base pair synthetic gene sequence was divided into 11 oligonucleotide fragments. Individual fragments were synthesized and cloned, either single or double stranded, into either pTZ 19R, pTZ 18R or pBluescript, amplified and fused. The synthetic OvIFNτ construct was then cloned into a modified pIN-III-ompA expression vector for expression in bacteria and also cloned into a yeast expression plasmid. A similarly constructed human IFNτ synthetic gene (SEQ ID NO:3) has been designed, constructed and expressed in yeast cells.

Expression of the OvIFNτ synthetic gene in yeast (Example 4) allowed over production of recombinant IFNτ in S. cerevisiae: large quantities (5-20 mg/l) of recombinant IFNτ can be purified from soluble yeast extract using sequential ion exchange and molecular sieve chromatography. Recombinant IFNτ purified in this fashion exhibited potent antiviral activity (2 to 3 $\times$ 10^8 units/mg) similar to native OvIFNτ.

The synthetic gene construct facilitates introduction of mutations for possible enhancement of antitumor (anticellular proliferative) and antiviral activities. Further, the disparate regions of the molecule responsible for different functions can be modified independently to generate a molecule with a desired function. For example, two deletion mutants, OvIFNτ(1-162) and OvIFNτ(1-166), have been constructed to examine the role of carboxy terminal sequences in IFNτ molecules.

Additional mutant IFNτ molecules have been constructed to identify residues critical for antiproliferative activity. For example, one particular residue, TYR 123 has been implicated in the anticellular proliferative activity of IFNα (McInnes, et al., 1989). The equivalent of TYR 123 in IFNτ is contained within peptide OvIFNτ(119-150): this polypeptide inhibits OvIFNτ and human IFNα antiproliferative activity. Mutations converting TYR 123 to conservative (TRP) and nonconservative (ASP) substitutions have been generated, as well as mutant sequences having deletion of this residue. The codon for TYR 123 is located within an SspI site; elimination of this

site has been used for screening. The antiproliferative activity of these mutant IFNτ is evaluated
as described herein.

Synthetic peptides can be generated which correspond to the IFNτ polypeptides of the present
invention. Synthetic peptides can be commercially synthesized or prepared using standard methods
5   and apparatus in the art (Applied Biosystems, Foster City CA).

Alternatively, oligonucleotide sequences encoding peptides can be either synthesized directly
by standard methods of oligonucleotide synthesis, or, in the case of large coding sequences,
synthesized by a series of cloning steps involving a tandem array of multiple oligonucleotide
fragments corresponding to the coding sequence (Crea, 1989; Yoshio, et al., 1989; Eaton, et al.,
10   1988). Oligonucleotide coding sequences can be expressed by standard recombinant procedures
(Maniatis et al., 1982; Ausubel et al., 1988).

The biological activities of the interferon-τ polypeptides described above can be exploited
using either the interferon-τ polypeptides alone or conjugated or fused with other proteins as
described above and below.

15   V. Production of Fusion Proteins.

In another aspect, the present invention includes interferon-τ, interferon-τ-derived
polypeptides or hybrid interferons covalently attached to a second polypeptide to form a fused, or
hybrid, protein. The interferon-τ sequences making up such fused proteins can be recombinantly
produced interferon-τ or a bioactive portion thereof, as described above.

20   For example, where interferon-τ is used to inhibit viral expression, polypeptides derived from
IFNτ demonstrating antiviral activity may be advantageously fused with a soluble peptide, such
as, serum albumin, an antibody (e.g., specific against an virus-specific cell surface antigen), or
an interferon alpha polypeptide. In one embodiment, described above with reference to hybrid
interferon fusion polypeptides, the IFNτ polypeptides provide a method of reducing the toxicity
25   of other interferon molecules (e.g., IFNβ or IFNα) by replacing toxicity-associated regions of such
interferons with corresponding interferon-τ regions having lower toxicity. In another embodiment,
fusion proteins are generated containing interferon-τ regions that have anticellular proliferation
properties. Such regions may be obtained from, for example, the human interferon-τ sequences
disclosed herein.

30   The fused proteins of the present invention may be formed by chemical conjugation or by
recombinant techniques. In the former method, the interferon and second selected polypeptide are
modified by conventional coupling agents for covalent attachment. In one exemplary method for
coupling soluble serum albumin to an interferon polypeptide, serum albumin is derivatized with

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N-succinimidyl-S-acetyl thioacetate (Duncan, *et al.*, 1983), yielding thiolated serum albumin. The activated serum albumin polypeptide is then reacted with interferon derivatized with N-suc-cinimidyl 3-(2-pyridyldithio) propionate (Cumber, *et al.*, 1985), to produce the fused protein joined through a disulfide linkage.

5   As an alternative method, recombinant interferon may be prepared with a cysteine residue to allow disulfide coupling of the interferon to an activated ligand, thus simplifying the coupling reaction. An interferon-τ expression vector, used for production of recombinant interferon-τ, can be modified for insertion of an internal or a terminal cysteine codon according to standard methods of site-directed mutagenesis (Ausubel, *et al.*, 1988).

10   In one method, a fused protein is prepared recombinantly using an expression vector in which the coding sequence of a second selected polypeptide is joined to the interferon-τ coding sequence. For example, human serum albumin coding sequences can be fused in-frame to the coding sequence of an interferon-τ polypeptide, such as, SEQ ID NO:9, SEQ ID NO:19 or SEQ ID NO:39. The fused protein is then expressed using a suitable host cell. The fusion protein may

15   be purified by molecular-sieve and ion-exchange chromatography methods, with additional purifi-cation by polyacrylamide gel electrophoretic separation and/or HPLC chromatography, if neces-sary.

It will be appreciated from the above how interferon-τ-containing fusion proteins may be prepared. One variation on the above fusion is to exchange positions of the interferon-τ and

20   selected second protein molecules in the fusion protein (*e.g.*, carboxy terminal versus amino termi-nal fusions). Further, internal portions of a native interferon-τ polypeptide (for example, amino acid regions of between 15 and 172 amino acids) can be assembled into polypeptides where two or more such interferon-τ portions are contiguous that are normally discontinuous in the native protein.

25   VI.   Antibodies Reactive with Interferon-τ.

Fusion proteins containing the polypeptide antigens of the present invention fused with the glutathione-S-transferase (Sj26) protein can be expressed using the pGEX-GLI vector system in *E. coli* JM101 cells. The fused Sj26 protein can be isolated readily by glutathione substrate affinity chromatography (Smith, D.B., *et al.*, 1988). Expression and partial purification of IFN

30   proteins is described in (Example 21), and is applicable to any of the soluble, induced polypeptides coded by sequences described by the present invention.

Insoluble GST (sj26) fusion proteins can be purified by preparative gel electrophoresis.

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Alternatively, IFNτ-β-galactosidase fusion proteins can be isolated as described in Example 20.

Also included in the invention is an expression vector, such as the lambda gt11 or pGEX vectors described above, containing IFNτ coding sequences and expression control elements which
5   allow expression of the coding regions in a suitable host. The control elements generally include a promoter, translation initiation codon, and translation and transcription termination sequences, and an insertion site for introducing the insert into the vector.

The DNA encoding the desired polypeptide can be cloned into any number of vectors (discussed above) to generate expression of the polypeptide in the appropriate host system. These
10 recombinant polypeptides can be expressed as fusion proteins or as native proteins. A number of features can be engineered into the expression vectors, such as leader sequences which promote the secretion of the expressed sequences into culture medium. Recombinantly produced IFNs, and polypeptides derived therefrom, are typically isolated from lysed cells or culture media. Purification can be carried out by methods known in the art including salt fractionation, ion exchange
15 chromatography, and affinity chromatography. Immunoaffinity chromatography can be employed using antibodies generated against selected IFNτ or hybrid interferon antigens.

In another aspect, the invention includes specific antibodies directed against the polypeptides of the present invention. Typically, to prepare antibodies, a host animal, such as a rabbit, is immunized with the purified antigen or fused protein antigen. Hybrid, or fused, proteins may be
20 generated using a variety of coding sequences derived from other proteins, such as β-galactosidase or glutathione-S-transferase. The host serum or plasma is collected following an appropriate time interval, and this serum is tested for antibodies specific against the antigen. Example 21 describes the production of rabbit serum antibodies which are specific against the hybIFN antigens in a Sj26/hybIFN hybrid protein. These techniques can be applied to the all of the hybIFN molecules
25 and polypeptides derived therefrom.

The gamma globulin fraction or the IgG antibodies of immunized animals can be obtained, for example, by use of saturated ammonium sulfate or DEAE Sephadex, or other techniques known to those skilled in the art for producing polyclonal antibodies.

Alternatively, purified protein or fused protein may be used for producing monoclonal
30 antibodies. Here the spleen or lymphocytes from a animal immunized with the selected polypeptide antigen are removed and immortalized or used to prepare hybridomas by methods known to those skilled in the art (Harlow, et al., 1988). Lymphocytes can be isolated from a peripheral blood sample. Epstein-Barr virus (EBV) can be used to immortalize human lymphocytes or a fusion partner can be used to produce hybridomas.

Antibodies secreted by the immortalized cells are screened to determine the clones that secrete antibodies of the desired specificity, for example, by using the ELISA or Western blot method (Ausubel *et al.*, 1988). Experiments performed in support of the present invention have yielded four hybridomas producing monoclonal antibodies specific for ovine IFNτ have been isolated.

5   Antigenic regions of polypeptides are generally relatively small, typically 7 to 10 amino acids in length. Smaller fragments have been identified as antigenic regions. Interferon-τ polypeptide antigens are identified as described above. The resulting DNA coding regions can be expressed recombinantly either as fusion proteins or isolated polypeptides.

In addition, some amino acid sequences can be conveniently chemically synthesized (Applied
10   Biosystems, Foster City CA). Antigens obtained by any of these methods may be directly used for the generation of antibodies or they may be coupled to appropriate carrier molecules. Many such carriers are known in the art and are commercially available (*e.g.*, Pierce, Rockford IL).

Antibodies reactive with IFNτ or hybrid interferons are useful, for example, in the analysis of structure/function relationships.

15   VII. Utility

        A.   Reproductive.

Although IFNτ bears some similarity to the IFNα family based on structure and its potent antiviral properties, the IFNαs do not possess the reproductive properties associated with IFNτ. For example, recombinant human IFNα had no effect on interestrous interval compared to IFNτ,
20   even when administered at twice the dose (Davis, *et al.*, 1992).

Therefore, although IFNτ has some structural similarities to other interferons, it has very distinctive properties of its own: for example, the capability of significantly influencing the biochemical events of the estrous cycle.

The human IFNτ of the present invention can be used in methods of enhancing fertility and
25   prolonging the life span of the *corpus luteum* in female mammals as generally described in Hansen, *et al.*, 1991, herein incorporated by reference. Further, the human interferon-τ of the present invention could be used to regulate growth and development of uterine and/or fetal-placental tissues. The human IFNτ is particularly useful for treatment of humans, since potential antigenic responses are less likely using such a same-species protein.

30   B.   Antiviral Properties.

Type I interferons exhibit potent antiviral properties. The antiviral activity of IFNτ has broad therapeutic applications without the toxic effects that are usually associated with IFNαs. Although the presence of IFNτ in culture medium inhibited reverse transcriptase activity of the feline

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immunodeficiency virus (Example 11), this is not due to a direct effect of IFNτ on the reverse transcriptase. Rather, IFNτ appears to induce the host cell to produce a factor(s) which is inhibitory to the reverse transcriptase of the virus.

IFNτ was found to exert its antiviral activity without adverse effects on the cells: no evidence

5   of cytotoxic effects attributable to the administration of IFNτ was observed. It is the lack of cytotoxicity of IFNτ which makes it extremely valuable as an *in vivo* therapeutic agent. This lack of cytotoxicity sets IFNτ apart from most other known antiviral agents and all other known interferons.

Formulations comprising IFNτ-containing hybrid interferon fusion compounds of the present

10   invention can be used to inhibit viral replication.

The hybrid interferon fusion polypeptides of the present invention can be employed in methods for affecting the immune relationship between fetus and mother, for example, in preventing transmission of maternal viruses (*e.g.*, HIV) to the developing fetus. The human interferon compositions is particularly useful for treatment of humans, since potential antigenic

15   responses are less likely using a homologous protein.

C.   Anticellular Proliferation Properties.

Type I interferons exhibit potent anticellular proliferation activity. Hybrid interferons such as described herein can also be used to inhibit cellular growth without the negative side effects associated with other interferons which are currently known. Formulations comprising the hybrid

20   interferon compounds of the subject invention can be used to inhibit, prevent, or slow tumor growth.

The development of certain tumors is mediated by estrogen. Experiments performed in support of the present invention indicate that IFNτ can suppress estrogen receptor numbers. Therefore, IFNτ-containing compositions can be used in the treatment or prevention of estrogen-

25   dependent tumors.

D.   Immune System Disorders

Diseases which may be treated using methods of the present invention include autoimmune, inflammatory, proliferative and hyperproliferative diseases, as well as cutaneous manifestations of immunologically mediated diseases. In particular, methods of the present invention ᴿe

30   advantageous for treating conditions relating to immune system hypersensitivity. There are four types of immune system hypersensitivity (Clayman). Type I, or immediate/anaphylactic hypersensitivity, is due to mast cell degranulation in response to an allergen (*e.g.*, pollen), and includes asthma, allergic rhinitis (hay fever), urticaria (hives), anaphylactic shock, and other illnesses of an allergic nature. Type II, or autoimmune hypersensitivity, is due to antibodies that

are directed against perceived "antigens" on the body's own cells. Type III hypersensitivity is due to the formation of antigen/antibody immune complexes which lodge in various tissues and activate further immune responses, and is responsible for conditions such as serum sickness, allergic alveolitis, and the large swellings that sometimes form after booster vaccinations. Type IV

5   hypersensitivity is due to the release of lymphokines from sensitized T-cells, which results in an inflammatory reaction. Examples include contact dermatitis, the rash of measles, and "allergic" reactions to certain drugs.

The mechanisms by which certain conditions may result in hypersensitivity in some individuals are generally not well understood, but may involve both genetic and extrinsic factors.

10   For example, bacteria, viruses or drugs may play a role in triggering an autoimmune response in an individual who already has a genetic predisposition to the autoimmune disorder. It has been suggested that the incidence of some types of hypersensitivity may be correlated with others. For example, it has been proposed that individuals with certain common allergies are more susceptible to autoimmune disorders.

15   Autoimmune disorders may be loosely grouped into those primarily restricted to specific organs or tissues and those that affect the entire body. Examples of organ-specific disorders (with the organ affected) include multiple sclerosis (myelin coating on nerve processes), type I diabetes mellitus (pancreas), Hashimotos thyroiditis (thyroid gland), pernicious anemia (stomach), Addison's disease (adrenal glands), myasthenia gravis (acetylcholine receptors at neuromuscular

20   junction), rheumatoid arthritis (joint lining), uveitis (eye), psoriasis (skin), Guillain-Barré Syndrome (nerve cells) and Grave's disease (thyroid). Systemic autoimmune diseases include systemic lupus erythematosus and dermatomyositis.

Other examples of hypersensitivity disorders include asthma, eczema, atopical dermatitis, contact dermatitis, other eczematous dermatitides, seborrheic dermatitis, rhinitis, Lichen planus,

25   Pemplugus, bullous Pemphigoid, Epidermolysis bullosa, uritcaris, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Alopecia areata, atherosclerosis, primary biliary cirrhosis and nephrotic syndrome. Related diseases include intestinal inflammations, such as Coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, inflammatory bowel disease, Chrohn's disease and ulcerative colitis, as well as food-related allergies.

30   Autoimmune diseases particularly amenable for treatment using the methods of the present invention include multiple sclerosis, type I (insulin dependent) diabetes mellitus, lupus erythematosus, amyotrophic lateral sclerosis, Crohn's disease. rheumatoid arthritis, stomatitis, asthma, uveitis, allergies and psoriasis.

Medicaments containing hybIFN may be used to therapeutically treat and thereby alleviate symptoms of autoimmune disorders such as those discussed above.

E. Interfering with the Binding of Interferons to Receptors.

IFNτ appears to interact with the Type I IFN receptor via several epitopes on the molecule,
5 and these regions either separately or in combination may affect distinct functions of IFNτ differently.

The polypeptides of the present invention are useful for the selective inhibition of binding of interferons to the interferon receptor. Specifically, as described herein, certain of the disclosed peptides selectively inhibit the antiviral activity of IFNτ while others inhibit the antiproliferative
10 activity. Combinations of these peptides could be used to inhibit both activities. Advantageously, despite binding to the interferon receptor and blocking IFNτ activity, these peptides do not, themselves, elicit the antiviral or antiproliferative activity.

Therefore, such polypeptides can be used as immunoregulatory molecules when it is desired to prevent immune responses triggered by interferon molecules. These peptides could be used as
15 immunosuppressants to prevent, for example, interferon-mediated immune responses to tissue transplants. Other types of interferon mediated responses may also be blocked, such as the cytotoxic effects of alpha interferon.

F. Pharmaceutical Compositions.

Hybrid interferon fusion polypeptides of the present invention can be formulated according
20 to known methods for preparing pharmaceutically useful compositions. Formulations comprising interferons or interferon-like compounds have been previously described (for example, Martin, 1976). In general, the compositions of the subject invention will be formulated such that an effective amount of the hybrid interferon is combined with a suitable carrier in order to facilitate effective administration of the composition.

25 The compositions used in these therapies may also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspensions, liposomes, suppositories, injectable, and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and
30 adjuvants which are known to those of skill in the art. Preferably, the compositions of the invention are in the form of a unit dose and will usually be administered to the patient one or more times a day.

Hybrid interferon fusion polypeptides or related polypeptides, may be administered to a patient in any pharmaceutically acceptable dosage form, including oral intake, inhalation, intranasal

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spray, intraperitoneal, intravenous, intramuscular, intralesional, or subcutaneous injection. Specifically, compositions and methods used for other interferon compounds can be used for the delivery of these compounds.

One primary advantage of the compounds of the subject invention, however, is the extremely
5   low cytotoxicity of IFNτ proteins. Because of this low cytotoxicity, it is possible to administer the hybrid interferon compositions in concentrations which are greater than those which can generally be utilized for other interferon (e.g., IFNα) compounds. Thus, it is contemplated that hybrid interferon compositions of the present invention can be administered at rates from about 5×10^4 to 20×10^6 units/day to about 500×10^6 units/day or more. In a preferred embodiment,
10   the dosage is about 20×10^6 units/day. High doses are preferred for systemic administration. It should, of course, be understood that the compositions and methods of this invention may be used in combination with other therapies.

Once improvement of a patient's condition has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be
15   reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

The compositions of the subject invention can be administered through standard procedures
20   to treat a variety of cancers and viral diseases including those for which other interferons have previously shown activity. See, for example, Finter, et al., 1991; Dianzani, 1992; Francis, et al., 1992, and U.S. Patent Nos. 4,885,166 and 4,975,276. However, as discussed above, the compositions of the subject invention have unique features and advantages, including their ability to treat these conditions without toxicity.

25   G.   Treatment of Skin Disorders.

Disorders of the skin can be treated intralesionally using hybrid interferons of the present invention, wherein formulation and dose will depend on the method of administration and on the size and severity of the lesion to be treated. Preferred methods include intradermal and subcutaneous injection. Multiple injections into large lesions may be possible, and several lesions
30   on the skin of a single patient may be treated at one time. The schedule for administration can be determined by a person skilled in the art. Formulations designed for sustained release can reduce the frequency of administration.

H.   Systemic Treatment.

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Systemic treatment is essentially equivalent for all applications. Multiple intravenous, subcutaneous and/or intramuscular doses are possible, and in the case of implantable methods for treatment, formulations designed for sustained release are particularly useful. Patients may also be treated using implantable subcutaneous portals, reservoirs, or pumps.

5   I.   Regional Treatment.

Regional treatment with the hybrid interferons fusion polypeptides of the present invention is useful for treatment of cancers in specific organs. Treatment can be accomplished by intra-arterial infusion. A catheter can be surgically or angiographically implanted to direct treatment to the affected organ. A subcutaneous portal, connected to the catheter, can be used for chronic

10   treatment, or an implantable, refillable pump may also be employed.

The following examples illustrate, but in no way are intended to limit the present invention.

Materials and Methods

Restriction endonucleases, T4 DNA ligase, T4 polynucleotide kinase, *Taq* DNA polymerase, and calf intestinal phosphatase were purchased from New England Biolabs (Beverly, MA) or

15   Promega Biotech (Madison, WI): these reagents were used according to the manufacturer's instruction. For sequencing reactions, a "SEQUENASE DNA II" sequencing kit was used (United States Biochemical Corporation, Cleveland OH). Immunoblotting and other reagents were from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Needham, MA). Nitrocellulose filters are obtained from Schleicher and Schuell (Keene, NH).

20   Synthetic oligonucleotide linkers and primers are prepared using commercially available automated oligonucleotide synthesizers (*e.g.*, an ABI model 380B-02 DNA synthesizer (Applied Biosystems, Foster City, CA)). Alternatively, custom designed synthetic oligonucleotides may be purchased, for example, from Synthetic Genetics (San Diego, CA). cDNA synthesis kit and random priming labeling kits are obtained from Boehringer-Mannheim Biochemical (BMB,

25   Indianapolis, IN).

Oligonucleotide sequences encoding polypeptides can be either synthesized directly by standard methods of oligonucleotide synthesis, or, in the case of large coding sequences, synthesized by a series of cloning steps involving a tandem array of multiple oligonucleotide fragments corresponding to the coding sequence (Crea, 1989; Yoshio, *et al.*, 1989; Eaton, *et al.*,

30   1988). Oligonucleotide coding sequences can be expressed by standard recombinant procedures (Maniatis, *et al.*, 1982; Ausubel *et al.*, 1988). Alternatively, peptides can be synthesized directly by standard *in vitro* techniques (Applied Biosystems, Foster City CA).

Recombinant human IFNα (rHuIFNα) and rBoIFNγ was obtained from Genentech Inc. (South San Francisco, CA). The reference preparation of recombinant human IFNα (rHuIFNα) was obtained from the National Institutes of Health: rHuIFNα is commercially available from Lee Biomolecular (San Diego, CA). Purified recombinant human IFNαA (2×10^8 units/mg) was

5 obtained from Biosource International (Camarillo, CA). Unless otherwise indicated, protein concentration was determined with the bicinchoninic acid assay kit (Pierce, Rockford IL) according to the manufacturer's instructions.

Common manipulations involved in polyclonal and monoclonal antibody work, including antibody purification from sera, are performed by standard procedures (Harlow, *et al.*, 1988).

10 Pierce (Rockford, IL) is a source of many antibody reagents.

Affinity-purified rabbit antipeptide antibodies specific to Tyk2, Statlα, and Stat2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antiphosphotyrosine antibody (4G10) was obtained from Upstate Biotechnology (Lake Placid, NY). Western blots were developed with an enhanced chemiluminescence (ECL) detection kit (Amersham Corp., Arlington

15 Heights, IL).

The bovine kidney cell line MDBK and the human Burkitt lymphoma cell line Daudi were obtained from the American Type Culture Collection (ATCC; Rockville, MD). The Daudi cells were grown in RPMI 1640 medium supplemented with 20% fetal bovine serum and antibiotics (Gibco/BRL, Gaithersburg, MD). MDBK cells were grown in Eagle's minimal essential medium

20 (EMEM) supplemented with 10% horse serum and antibiotics (Gibco/BRL).

All tissue culture media, sera and IFNs used in this study were negative for endotoxin, as determined by assay with Limulus amebocyte lysate (Associates of Cape Cod, Woods Hole, MA) at a sensitivity level of 0.07 ng/ml.

General ELISA Protocol for Detection of Antibodies.

25 Polystyrene 96 well plates Immulon II (PGC) were coated with 5 μg/mL (100 μL per well) antigen in 0.1 M carb/bicarbonate buffer, pH 9.5. Plates were sealed with parafilm and stored at 4°C overnight.

Plates were aspirated and blocked with 300 uL 10% NGS and incubated at 37°C for 1 hr. Plates were washed 5 times with PBS 0.5% "TWEEN-20".

30 Antisera were diluted in 0.1 M PBS, pH 7.2. The desired dilution(s) of antisera (0.1 mL) were added to each well and the plate incubated 1 hours at 37°C. The plates was then washed 5 times with PBS 0.5% "TWEEN-20".

Horseradish peroxidase (HRP) conjugated goat anti-human antiserum (Cappel) was diluted 1/5,000 in PBS. 0.1 mL of this solution was added to each well. The plate was incubated 30 min at 37°C, then washed 5 times with PBS.

Sigma ABTS (substrate) was prepared just prior to addition to the plate.

5   The reagent consists of 50 mL 0.05 M citric acid, pH 4.2, 0.078 mL 30% hydrogen peroxide solution and 15 mg ABTS. 0.1 mL of the substrate was added to each well, then incubated for 30 min at room temperature. The reaction was stopped with the addition of 0.050 mL 5% SDS (w/v). The relative absorbance is determined at 410 nm.

EXAMPLE 1
Reproductive Functions of IFNτ

10   The effect of interferon-τ on the lifespan of the corpus lutem was examined.

IFNτ was infused into uterine lumen of ewes at the concentrations given in Table 1. Recombinant human IFNα (rHuIFNα) was infused at similar concentrations. In addition, control animals, which received control proteins, were also used. The life span of the corpus luteum was

15   assessed by examination of interestrous intervals, maintenance of progesterone secretion, and inhibition of prostaglandin secretion (Davis, et al., 1992).

Table 1
Effect of Interferons on Reproductive Physiology

Interferon	Treatment	Interestrous Interval (days) (Means)
Control	-	17.3
rHuIFNα	100 μg/day	16.0
	200 μg/day	16.0
	2000 μg/day	19.0
OvIFNτ	100 μg/day	27.2

Comparison of the interestrous intervals for the control animals and for animals receiving OvIFNτ demonstrate a considerable lengthening of the interval, when IFNτ is administered at

25   100μg/day. On the other hand, comparison of the interestrous interval for the control animal and for animals receiving recombinant human IFNα, demonstrated that rHuIFNα had no meaningful effect.

These results demonstrate that interferon-τ has the capability of significantly influencing the biochemical events of the reproductive cycle.

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EXAMPLE 2
Antiviral Properties of Interferon-τ at Various Stages of the Reproductive Cycle

Conceptus cultures were established using conceptus obtained from sheep at days 12 through 16 of the estrous cycle. Antiviral activity of supernatant from each conceptus culture was assessed

5   using a cytopathic effect assay (Familetti, et al., 1981). Briefly, dilutions of IFNτ or other IFNs were incubated with Madin-Darby bovine kidney (MDBK) cells for 16-18 hours at 37°C. Following incubation, inhibition of viral replication was determined in a cytopathic effect assay using vesicular stomatitis virus (VSV) as the challenge virus (Armstrong, 1981).

One antiviral unit caused a 50% reduction in destruction of the monolayer, relative to

10   untreated MDBK cells infected with VSV (control plates). Specific activities were further evaluated using normal ovine fibroblasts (Shnf) in a plaque inhibition assay (Langford, et al., 1981). A minimum of three samples were examined at each time point, and each sample was assayed in triplicate. The results presented in Table 2 are expressed as mean units/ml.

Table 2

15   IFNτ Antiviral Activity of Conceptus Cultures and Allantoic and Amniotic Fluids

	Day	Samples	Units/ml
Conceptus Cultures	10	9	<3
	12	5	34
	13	6	4.5×10^3
	14	3	7.7×10^3
	16	12	2.0×10^6
Allantoic Fluid	60	3	1.4×10^3
	100	4	11
	140	3	<3
Amniotic Fluid	60	3	22
	100	4	<3

Culture supernatants had increasing antiviral activity associated with advancing development

20   of the conceptus (Table 2).

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EXAMPLE 3
Expression of IFNτ in Bacteria

The amino acid coding sequence for OvIFNτ (Imakawa, et al., 1987) was used to generate a corresponding DNA coding sequence with codon usage optimized for expression in E. coli.

5   Linker sequences were added to the 5' and 3' ends to facilitate cloning in bacterial expression vectors. The nucleotide sequence was designed to include 19 unique restriction enzyme sites spaced evenly throughout the coding sequence (Figures 1A and 1B).

The nucleotide sequence was divided into eleven oligonucleotide fragments ranging in sizes of 33 to 75 bases. Each of the eleven oligonucleotides were synthesized on a 380-B 2-column

10   DNA synthesizer (Applied Biosystems) and cloned single- or double-stranded into one of the following vectors: "pBLUESCRIPT⁺(KS)" (Stratagene, LaJolla, CA), pTZ18R (Pharmacia, Piscataway, NJ), or pTZ19R (Pharmacia, Piscataway, NJ) cloning vectors.

The vectors were transformed into E. coli K. strain "XL1-BLUE" (recA1 endA1 gyrA96 thi hsdR17 (r, m, +) supE44 relA1 λ - (lac), {F', proAB, lacᵠZΔM15, Tn10(tetᴿ}) which is

15   commercially available from Stratagene (La Jolla, CA). Transformed cells were grown in L broth supplemented with ampicillin (50 μg/ml). Oligonucleotide cloning and fusion was performed using standard recombinant DNA techniques.

Cloning vectors were cut with the appropriate restriction enzymes to insert the synthetic oligonucleotides. The vectors were treated with calf intestine alkaline phosphatase (CIP) to

20   remove terminal phosphate groups. Oligonucleotides were phosphorylated and cloned, as either single- or double-stranded molecules, into the appropriate vector using T4 DNA ligase. When single-strands were introduced into cloning vectors, the second strand was completed by the bacterial host following transfection.

For double-stranded cloning, oligonucleotides were first annealed with their synthetic

25   complementary strand then ligated into the cloning vector. E. coli K12 strains SB221 or NM522 were then transformed with the ligation. E. coli strain GM119 was used for cloning when the methylation-sensitive StuI and ClaI restriction sites were involved. Restriction analyses were performed on isolated DNA at each stage of the cloning procedure.

Cloned oligonucleotides were fused into a single polynucleotide using the restriction diges-

30   tions and ligations outlined in Figure 2. Oligonucleotide-containing-DNA fragments were typically isolated after electrophoretic size fractionation on low-melting point agarose gels (Maniatis, et al., 1982; Sambrook, et al., 1989; Ausubel, et al., 1988). The resulting IFNτ polynucleotide coding sequence spans position 16 through 531: a coding sequence of 172 amino acids.

The nucleotide sequence of the final polynucleotide was confirmed by DNA sequencing using

35   the dideoxy chain termination method.

The full length *StuI/SstI* fragment (540 bp; Figure 2) was cloned into a modified pIN III omp-A expression vector and transformed into a competent SB221 strain of *E. coli*. For expression of the IFNτ protein, cells carrying the expression vector were grown in L-broth containing ampicillin to an OD (550 nm) of 0.1-1, induced with IPTG for 3 hours and harvested by
5   centrifugation. Soluble recombinant IFNτ was liberated from the cells by sonication or osmotic ractionation.

EXAMPLE 4
Expression of IFNτ in Yeast

The synthetic IFNτ gene, synthesized in Example 3, was flanked at the 5' end by an *StuI*
10   restriction site and at the 3' end by a *SacI* restriction site.

A.   Isolation of the Synthetic IFNτ Gene.

Two oligonucleotide primers (SEQ ID NO:13 and SEQ ID NO:14) were used to attach linkers to the synthetic IFNτ gene using polymerase chain reaction. The linker at the 5' end allowed the placement of the synthetic IFNτ gene in correct reading with the ubiquitin coding
15   sequence present in the yeast cloning vector pBS24Ub (Chiron Corp., Emeryville, CA). The linker also constructed a ubiquitin-IFNτ junction region that allowed *in vivo* cleavage of the ubiquitin sequences from the IFNτ sequences. The 5' oligonucleotide also encoded a *SacII* restriction endonuclease cleavage site. The 3' oligonucleotide contained a *StuI* cleavage site.

The vector carrying the synthetic IFNτ gene (Example 3) was isolated from *E. coli* strain
20   "XLI-BLUE" by the alkaline lysis method. Isolated vector was diluted 500-fold in 10 mM Tris, pH 8.0/1 mM EDTA/10 mM NaCl. The PCR reaction was performed in a 100 μl volume using *Taq* DNA polymerase and primers SEQ ID NO:13/SEQ ID NO:14. The amplified fragments were digested with *StuI* and *SacII*. These digested fragments were ligated into the *SacII* and *SmaI* sites of "pBLUESCRIPT+(KS)."
25   The resulting plasmid was named pBSY-IFNτ. The DNA sequence was verified using double stranded DNA as the template.

B.   Construction of the Expression Plasmid.

Plasmid pBSY-IFNτ was digested with *SacII* and *EcoRV* and the fragment containing the synthetic IFNτ gene was isolated. The yeast expression vector pBS24Ub (Sabin, *et al.*, 1989;
30   Ecker, *et al.*, 1989) was digested with *SalI*. Blunt ends were generated using T4 DNA polymerase. The vector DNA was extracted with phenol and ethanol precipitated (Sambrook, *et al.*, 1989). The recovered linearized plasmid was digested with *SacII*, purified by agarose gel

electrophoresis, and ligated to the *SacII-Eco*RV fragment isolated from pBSY-IFNτ. The resulting recombinant plasmid was designated pBS24Ub-IFNτ.

The recombinant plasmid pBS24Ub-IFNτ was transformed into *E. coli*. Recombinant clones containing the IFNτ insert were isolated and identified by restriction enzyme analysis. Plasmid

5   DNA from clones containing IFNτ coding sequences was used for transformation of *S. cerevisiae* (Rothstein, 1986). Transformation mixtures were plated on uracil omission medium and incubated for 3-5 days at 30°C. Colonies were then streaked and maintained on uracil and leucine omission medium (Rothstein, 1986).

   C.   Expression Experiments.

10 For small-scale expression, a single colony of *S. cerevisiae* AB116 containing pBS24Ub-IFNτ was picked from a leucine and uracil omission plate and grown at 30°C in YEP medium (1% yeast extract, 2% peptone) containing 1% glucose for inducing conditions or 8% glucose for non-inducing conditions. Cell lysates were recovered and subjected to SDS-PAGE in 15% acrylamide, 0.4% bisacrylamide (Sambrook, *et al.*, 1989). The fractionated proteins were visualized by

15 Coomassie blue staining.

   Recombinant IFNτ was visualized specifically by immunoblotting with monoclonal antibody or polyclonal antiserum against ovine IFNτ upon electrotransfer of the fractionated cell extract to "NYTRAN" paper (Rothstein, 1986).

   For large-scale expression, pBS24-IFNτ was grown for 24 hours at 30°C in 5 × uracil and

20 leucine omission medium containing 8% glucose. This culture was then diluted 20-fold in YEP medium containing 1% glucose and further incubated for another 24-36 hours.

   Cells were harvested by centrifugation, washed in 50 mM Tris, pH 7.6,/1 mM EDTA and resuspended in wash buffer containing 1 mM PMSF. The cells were lysed using a Bead-beater apparatus (Biospec Products, Bartlesville, OK). The lysate was spun at 43,000 × g for 20

25 minutes. The supernatant fraction was recovered and subjected to the purification protocol described below.

   D.   Purification of roIFNτ from Yeast Cell Lysate.

   The supernatant was loaded on a 1 × 10 cm DEAE column and washed with 10 mM Tris, pH 8.0. Retained proteins were eluted with a 300 ml, 0 to 0.5 M NaCl gradient in 10 mM Tris,

30 pH 8.0. Three-milliliter fractions were collected. Ten-microliter samples of fractions 17-26 containing the recombinant (roIFNτ) were electrophoretically separated on 15% SDS-polyacryla-mide gels. The gels were stained with Coomassie blue.

   Fractions 18, 19, and 20 contained largest amount of roIFNτ. These fractions were loaded individually on a 1.5 × 90 cm Sephadex S-200 column and proteins were resolved in two peaks.

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Aliquots of each protein peak (25 μl) were electrophoretically separated on 15% SDS-poly-acrylamide gels and the proteins visualized with Coomassie staining.

Purified roIFNτ-containing fractions were combined and the amount of roIFNτ quantified by radioimmunoassay (Vallet, et al., 1988). Total protein concentration was determined by using the

5   Lowry protein assay (Lowry, et al., 1951).

Microsequencing of purified roIFNτ demonstrated identity with native IFNτ through the first 15 amino acids, confirming that the ubiquitin/roIFNτ fusion protein was correctly processed *in vivo*.

Purified roIFNτ exhibited 2 to 3 × 10^8 units of antiviral activity per milligram of protein (n

10   = 3 replicate plates) which is similar to the antiviral activity of IFNτ purified from conceptus-conditioned culture medium (2 × 10^8 U/mg).

EXAMPLE 5
Southern Blot Analysis of Human High Molecular Weight DNA

Human venous blood samples from healthy donors were collected in heparinized tubes and

15   peripheral blood lymphocytes were isolated by density-gradient centrifugation using a Ficoll-Isopaque gradient (1.077 g/ml) (Sigma Chemical Co.). High molecular weight (HMW) DNA was isolated from these cells (Sambrook, et al., 1989).

Two 10 μg samples of HMW DNA were digested with the restriction endonucleases *Hind*III or *Pst*I (Promega) for 2 hours at 37°C, and the DNA fragments electrophoretically separated in

20   a 0.8% agarose gel (Bio-Rad, Richmond, CA) at 75 volts for 8 hours. The DNA fragments were transferred onto a nylon membrane (IBI-International Biotechnologies, Inc., New Haven, CT). The membrane was baked at 80°C for 2 hours and incubated at 42°C for 4 hours in the following prehybridization solution: 5 × SSC (1 × SSC is 0.15 M NaCl and 0.15 M sodium citrate), 50% vol/vol formamide, 0.6% (wt/vol) SDS, 0.5% (wt/vol) nonfat dry milk, 20 mM Tris-HCl (pH

25   7.5), 4 mM EDTA, and 0.5 mg/ml single stranded herring sperm DNA (Promega).

The filter was then incubated in a hybridization solution (5 × SSC, 20% vol/vol formamide, 0.6% (wt/vol) SDS, 0.5% (wt/vol) nonfat dry milk, 20 mM Tris-HCl (pH 7.5), 4 mM EDTA, and 2 × 10^8 cpm/ml ^{32}P-labelled OvIFNτ cDNA (Imakawa, et al., 1987) for 18 hours at 42°C. The filter was washed at 42°C for 15 minutes with 2 × SSC and 0.1% (wt/vol) SDS and exposed

30   to X-ray film (XAR, Eastman Kodak, Rochester, NY) at -80°C for 48 hours in the presence of an intensifying screen.

Autoradiography detected a hybridization signal at approximately 3.4 kb in DNA digested with *Pst*I and a slightly smaller (≈ 3.0 kb) fragment in the *Hind*III digested DNA. These results indicate the presence of human DNA sequences complementary to the OvIFNτ cDNA probe.

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EXAMPLE 6
Isolation of Partial Sequence of Human IFN-τ cDNA by PCR

Two synthetic oligonucleotides (each 25-mer), corresponding to the nucleotides in the DNA sequence from 231 to 255 (contained in SEQ ID NO:13) and 566 to 590 (contained in SEQ ID

5   NO:14) of OvIFNτ cDNA (numbering relative to the cap site, Imakawa, *et al.*, 1987) were synthesized. These primers contained, respectively, cleavage sites for the restriction endonucleases *PstI* and *EcoRI*. SEQ ID NO:13 was modified to contain the *EcoRI* site, which begins at position 569.

DNA was isolated from approximately 1×10^5 plaque forming units (pfu) of the following two cDNA libraries: human term placenta (Clontech, Inc., Palo Alto, CA) and human term

10   cytotrophoblast (Dr. J.F. Strauss, University of Pennsylvania, Philadelphia PA). The DNA was employed in polymerase chain reaction (PCR) amplifications (Mullis, 1987; Mullis, *et al.*, 1987; Perkin Elmer Cetus Corp. Norwalk CT). Amplification reactions were carried out for 30 cycles (45°C, 1m; 72°C, 2m; 94°C, 1m) (thermal cycler and reagents, Perkin Elmer Cetus) using primers SEQ ID NO:13/SEQ ID NO:14.

15   Amplification products were electrophoretically separated (100 volts in a 1.5% agarose gel (Bio-Rad)) and transferred onto a nylon membrane (IBI). The membrane was baked at 80°C for 2 hours and prehybridized and hybridized with ^{32}P-labelled OvIFNτ cDNA as described above. The membrane was washed in $5 \times$ SSC/0.1% (wt/vol) SDS for 5 minutes at 42°C and in $2 \times$ SSC/0.1% (wt/vol) SDS for 2 minutes at 42°C. It was then exposed at -80°C to "XAR" (Eastman

20   Kodak) X-ray film for 24 hours in the presence of an intensifying screen. An amplification product that hybridized with the labelled probe DNA was detected.

PCR was performed again as directed above. Amplified products were digested with the restriction endonucleases *EcoRI* and *PstI* (Promega) for 90 minutes at 37°C. The resulting DNA fragments were electrophoretically separated as described above and the band containing the IFNτ

25   amplification product was excised from the gel. DNA fragments were recovered by electroelution, subcloned into *EcoRI/PstI* digested-dephosphorylated plasmid pUC19 and transformed into *E. coli* strain JM101 (Promega) by calcium chloride method (Sambrook, *et al.*, 1989). The plasmids were isolated and the inserted amplification product sequenced using the dideoxy termination method (Sanger, *et al.*, 1977; "SEQUENASE" reactions, United States Biochemical, Cleveland, OH).

30   Nucleotide sequences were determined, and comparison of these as well as the deduced amino acid sequences to other IFN sequences were performed using "DNA STAR SOFTWARE" (Madison, WI).

Comparison of the sequences of these clones revealed the following four different clones: from the human placental library, HuIFNτ6 (299 bp), HuIFNτ7 (288 bp) and HuIFNτ4 (307 bp),

35   which exhibit 95% identity in their nucleotide sequences; from the cytotrophoblast library clone

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CTB 35 (HuIFNτ5; 294 basepairs), which shares 95% and 98% identity with HuIFNτ6 and HuIFNτ4, respectively.

EXAMPLE 7
Isolation of Full-Length Human IFNτ Genes

5   Ten micrograms PBMC HMW DNA was digested with restriction endonuclease *EcoRI* and subjected to electrophoretic analysis in a 0.8% agarose gel. A series of samples containing ranges of DNA fragments sized 1.5 to 10 kb (*e.g.*, 1.5 to 2.5 kb, 2.5 kb to 3 kb) were excised from the gel. The DNAs were electroeluted and purified. Each DNA sample was amplified as described above using the OvIFNτ primers. The DNA molecules of any sample that yielded a positive PCR signal were cloned into λgt11 (the subgenomic λgt11 library).

10

A.   PCR Identification of Clones Containing Sequences Complementary to OvIFNτ.

The λgt11 phage were then plated for plaques and plaque-lift hybridization performed using the ³²P-labelled OvIFNτ cDNA probe. Approximately 20 clones were identified that hybridized to the probe.

15   Plaques that hybridized to the probe were further analyzed by PCR using the OvIFNτ primers described above. Six plaques which generated positive PCR signals were purified. The phage DNA from these clones was isolated and digested with *EcoRI* restriction endonuclease. The DNA inserts were subcloned into pUC19 vectors and their nucleotide sequences determined by dideoxy nucleotide sequencings.

20   B.   Hybridization Identification of Clones Containing Sequences Complementary to PCR-Positive Phage.

Recombinant phage from the λgt11 subgenomic library were propagated in *E. coli* Y1080 and plated with *E. coli* Y1090 at a density of about 20,000 plaques/150 mm plate. The plates were overlaid with duplicate nitrocellulose filters, which were hybridized with a ³²P-labelled probe from one of the six human IFNτ cDNA clones isolated above.

25

Clones giving positive hybridization signals were further screened and purified. The phage DNAs from hybridization-positive clones were isolated, digested with *EcoRI*, subcloned into pUC19 vector and sequenced. The sequence information was then analyzed.

1.   HuIFNτ1

30   Three clones yielded over-lapping sequence information for over 800 bases relative to the mRNA cap site (clones were sequenced in both orientations). The combined nucleic acid sequence information is presented as SEQ ID NO:11 and the predicted protein coding sequence is presented as SEQ ID NO:12. Comparison of the predicted mature protein sequence (SEQ ID NO:12) of this gene to the predicted protein sequence of OvIFNτ is shown in Figure 3.

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2. HuIFNτ2, HuIFNτ3

Two additional clones giving positive hybridization signals (HuIFNτ2 and HuIFNτ3) were also screened, purified, and phage DNAs subcloned and sequenced as above. The sequences of these two clones are presented in Figures 19A and 19B. As can be appreciated in Figures 19A

5   and 19B, the nucleotide sequence of both clones (HuIFNτ2 and HuIFNτ3) is homologous to that of HuIFNτ1 and OvIFNτ.

HuIFNτ2 (SEQ ID NO:29), may be a pseudo-gene, as it appears to contain a stop codon at position 115-117. The sequence, SEQ ID NO:29, is presented without the leader sequence. The leader sequence is shown in Figure 20A. As can be seen from the HuIFNτ2 sequence presented

10   in Figure 20A, the first amino acid present in mature HuIFNτ1 (a CYS residue) is not present in the HuIFNτ2 sequence. Accordingly, the predicted amino acid sequence presented as SEQ ID NO:29 corresponds to a mature IFNτ protein with the exceptions of the first CYS residue and the internal stop codon.

The internal stop codon in the nucleic acid coding sequence can be modified by standard

15   methods to replace the stop codon with an amino acid codon, for example, encoding GLN. The amino acid GLN is present at this position in the other isolates of human IFNτ (HuIFNτ). Standard recombinant manipulations also allow introduction of the initial CYS residue if so desired.

HuIFNτ3 (SEQ ID NO:31), appears to encode a human IFNτ protein. The translated amino

20   acid sequence of the entire protein, including the leader sequence, is presented as SEQ ID NO:32. The translated amino acid sequence of the mature protein is presented as SEQ ID NO:34.

EXAMPLE 8
Analysis of the Presence of HuIFNτ mRNA by RT-PCR

Human placental cDNA libraries and an ovine cDNA library, constructed from day 15-16

25   conceptuses, were analyzed by hybridization to the OvIFNτ cDNA probe, described above. cDNAs were size-fractionated on agarose gels and transferred to filters (Maniatis, *et al.*, 1982; Sambrook, *et al.*, 1989). Southern blot analysis with OvIFNτ probe showed that the autoradiographic signals from human cDNA libraries were approximately 1/100 of the signal obtained using the OvIFNτ cDNA library.

30   The presence of HuIFNτ mRNA in human term placenta and amniocytes (26 weeks, 2 million cells) was analyzed by using reverse transcriptase-PCR (RT-PCR) method (Clontech Laboratories, Palo Alto CA).

Total cellular RNA (tcRNA) isolated from human placenta, amniocytes and ovine conceptuses were reverse transcribed using the primer SEQ ID NO:14. The primer SEQ ID NO:13 was then

55

added to the reaction and polymerase chain reaction carried out for 40 cycles. The PCR products were size fractionated on agarose gels and transferred to filters. The DNA on the filters was hybridized with ^{32}P-labelled OvIFNτ and HuIFNτ cDNAs. The results of these analyses demonstrate the presence of human IFNτ mRNA in the feto-placental annex. The aminocytes also

5   expressed the messages corresponding to OvIFNτ primers and human probe.

In addition, a RT-PCR analysis for the presence of HuIFNτ was applied to the tcRNA isolated from human adult lymphocytes. A densitometric analysis revealed that IFNτ mRNA exists in lymphocytes.

EXAMPLE 9
In Situ Hybridization

10

A.   Tissue

Slides of semiserial 5-μ paraffin embedded sections from four healthy, different term and first trimester human placentas were examined.

B.   cRNA Probe Preparation

15   From the cDNA clone isolated from OvIFNτ amplified library a fragment corresponding to the OvIFNτ cDNA bases #77-736 (base #1 is cap site; open reading frame of OvIFNτ cDNA is base #81-665; Figure 7) was subcloned into the transcription vector, pBS (New England Biolabs). Several pBS clones were isolated, subcloned, and their nucleotides sequenced. From this clone a 3' fragment (bases #425-736) was excised using the restriction endonucleases *Nla*IV and *Eco*RI

20   and subcloned into the transcription vector pBS. This vector was designated pBS/OvIFNτ.

After linearization of the pBS/OvIFNτ plasmid, an antisense cRNA probe was synthesized by *in vitro* transcription (Sambrook, *et al.*, 1989) using T₇ RNA polymerase (Stratagene). A trace amount of ^{3}H-CTP (NEN-DuPont, Cambridge, MA) was used in the transcription reaction. dUTP labeled with digoxigenin (Boehringer-Mannheim, Indianapolis, IN) was incorporated into the

25   cRNA and yield was estimated through TCA precipitation and scintillation counting.

C.   Hybridization

In situ hybridization was performed using the anti-sense RNA probe, as described by Lawrence, *et al.* (1985) with the following modifications. Deparaffinized and hydrated sections were prehybridized for 10 minutes at room temperature in phosphate buffered saline (PBS)

30   containing 5 mM MgCl₂. Nucleic acids in the sections were denatured for 10 minutes at 65°C in 50% formamide/2 × SSC. Sections were incubated overnight at 37°C with a hybridization cocktail (30 μl/slide) containing 0.3 μg/ml digoxigenin-labelled cRNA probe and then washed for 30 minutes each at 37°C in 50 formamide/1 × SSC. Final washes were performed for 30 minutes

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each at room temperature in 1 × SSC and 0.1 × SSC. The sections were blocked for 30 minutes with 0.5% Triton X-100 (Sigma) and 0.5% non-fat dry milk.

Hybridization signal was detected using purified sheep antidioxigenin Fab fragments conjugated to alkaline phosphatase (Boehringer-Mannheim). After unbound antibody was removed,

5   nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate substrate (Promega) and levamisole (Bector Laboratories, Burlingame, CA) were added for signal detection via colorimetric substrate generation. The tissues were counterstained in methyl green (Sigma), dehydrated, and mounted.

As a control, some tissue sections were pretreated with 100 μg/ml of pancreatic RNaseA (Sigma) for 30 minutes at 37°C. The RNase was inactivated on the slide with 400 units of RNase

10   inhibitor (Promega). The slides were then washed twice in 250 ml of PBS/5 mM MgCl$_2$. In other control experiments, tRNA (Sigma) was substituted for the digoxigenin probes.

Specific hybridization was observed in all term and first trimester placental tissues in three separate experiments with various OvIFNτ cRNA probe concentrations and blocking reagents.

First trimester placental villi composed of an outer layer of syncytiotrophoblast, an underlying

15   layer of cytotrophoblast, and a central stromal region with various types of mesenchymal cells, displayed the highest transcript level of IFNτ in the cytotrophoblast cells. Less intense but detectable levels were present in both the syncytiotrophoblast and stromal cells. A similar pattern of transcript expression was demonstrated in the placental villi of term tissue but the level of signal detection was low. First trimester extravillous trophoblast displayed the highest amount of

20   message and stained positive when present in the maternal blood spaces.

EXAMPLE 10
Antiviral Activity of IFNτ

The relative specific activity of OvIFNτ, purified to homogeneity, was evaluated in antiviral assays. The antiviral assays were performed essentially as described above in Example 2.

25   Specific activities are expressed in antiviral units/mg protein obtained from antiviral assays using either Madin-Darby bovine kidney (MDBK) cells or sheep normal fibroblasts (Shnf). All samples were assayed simultaneously to eliminate interassay variability. The results, presented in Table 3, are the means of four determinations where the standard deviation was less than 10% of the mean.

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Table 3
Antiviral Activity of IFNτ and Known IFNs

	Specific Activities	
	MDBK	Shnf
OvIFNτ	2×10^8	3×10^8
rBoIFNα	6×10^7	1×10^7
rBoIFNγ	4.5×10^6	3×10^6
NIH rHuIFNα	2.2×10^8	2.2×10^8
rHuIFNα	2.9×10^5	4.3×10^5

IFNτ had a higher specific activity than either rBoIFNα or rBoIFNγ (Table 3). The NIH standard preparation of rHuIFNα had a similar specific activity, while a commercial preparation of rHuIFNα exhibited low specific antiviral activity. Comparable relative antiviral activity was demonstrated using either bovine or ovine cells.

EXAMPLE 11
Anti-Retroviral Activity and Cytotoxic Effects of IFNτ

Highly purified OvIFNτ was tested for anti-retroviral and cytotoxic effects on feline peripheral blood lymphocytes exposed to the feline immunodeficiency retrovirus. This lentivirus produces a chronic AIDS-like syndrome in cats and is a model for human AIDS (Pederson, *et al.*, 1987). Replication of the virus in peripheral blood lymphocytes is monitored by reverse transcriptase activity in culture supernatants over time. The data from these assays are presented in Table 4.

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Table 4
Effect of OvIFNτ on FIV Replication

IFNτ Concentration (ng/ml)	RT Activity (cpm/ml)				
Experiment 1	**Harvest Days**				
	Day 2	Day 5	Day 8	Day 12	Day 15
0.00	93,908	363,042	289,874	171,185	125,400
0.62	77,243	179,842	172,100	218,281	73,039
1.25	94,587	101,873	122,216	71,916	50,038
2.50	63,676	72,320	140,783	75,001	36,105
5.00	69,348	82,928	90,737	49,546	36,299
Experiment 2	**Harvest Days**				
	Day 2	Day 5	Day 8	Day 13	Day 17
0.0	210,569	305,048	279,556	500,634	611,542
2.5	121,082	106,815	108,882	201,676	195,356
5.0	223,975	185,579	108,114	175,196	173,881
10.0	167,425	113,631	125,131	131,649	129,364
20.0	204,879	80,399	59,458	78,277	72,179
40.0	133,768	54,905	31,606	72,580	53,493

Addition of OvIFNτ produced a rapid, dose-dependent decrease in reverse transcriptase (RT) activity (Table 4). While concentrations as low as 0.62 ng/ml of IFNτ inhibited viral replication, much higher concentrations (40 ng/ml) having greater effects on RT-activity were without toxic effects on the cells. The results suggest that replication of the feline immunodeficiency virus was reduced significantly compared to control values when cells were cultured in the presence of OvIFNτ.

IFNτ appeared to exert no cytotoxic effect on the cells hosting the retrovirus. This was true even when IFNτ was present at 40 ng per ml of culture medium.

EXAMPLE 12
Effects of IFNτ on HIV Infected Human Peripheral Lymphocytes

IFNτ was also tested for activity against HIV infection in human cells. Human peripheral blood lymphocytes, which had been infected with HIV (Crowe, et al., 1987), were treated with

varying concentrations of OvIFNτ. Replication of HIV in peripheral blood lymphocytes was monitored by reverse transcriptase activity in culture supernatants over time. Reverse transcriptase activity was measured essentially by the method of Hoffman, et al. (1985). The data from these assays are presented in Table 5.

Table 5
Effect of OvIFNτ on HIV Replication
in Human Peripheral Lymphocytes

| IFNτ Concentration (ng/ml) | RT Activity | | | |
| | Day 6 | | Day 10 | |
	cpm/ml	% Reduction	cpm/ml	% Reduction
0	4,214	--	25,994	--
10	2,046	51	9,883	62
50	1,794	57	4,962	81
100	1,770	58	3,012	88
500	1,686	60	2,670	90
1000	1,499	64	2,971	89

As shown in Table 5, concentrations of OvIFNτ produced significant antiviral effects. A concentration of only 10 ng/ml resulted in over a 50% reduction in RT activity after only six days. A concentration of 500 ng/ml resulted in a 90% reduction in RT activity within 10 days.

The viability of human peripheral blood lymphocytes after treatment with IFNτ, over a range of concentrations for 3-13 days, was evaluated by trypan blue exclusion. The results of this viability analysis are presented in Table 6.

Table 6
Effect of OvIFNτ on Viability of
HIV Infected Human Peripheral Lymphocytes

| IFNτ Concentration (ng/ml) | Viable Cells/ml × 10^5 | | |
	Day 3	Day 6	Day 13
0	16.0	7.5	5.3
10	13.0	7.5	6.0
50	13.0	11.5	9.0
100	15.0	8.5	9.5
500	16.5	12.0	11.0

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IFNτ Concentration (ng/ml)	Viable Cells/ml × 10^5		
	Day 3	Day 6	Day 13
1000	21.9	9.5	8.5

The data presented in Table 6 show no evidence of cytotoxic effects attributable to the administration of IFNτ.

EXAMPLE 13
Inhibition of Cellular Growth

The effects of IFNτ on cellular growth were also examined. Anti-cellular growth activity was examined using a colony inhibition assay. Human amnion (WISH) or MDBK cells were plated at low cell densities to form colonies originating from single cells. Cells were cultured at 200 or 400 cells/well in 24 well plates in HMEM supplemented with 2% fetal bovine serum (FBS) and essential and nonessential amino acids. Various dilutions of interferons were added to triplicate wells, and the plates were incubated for 8 days to allow colony formation. Colonies were visualized after staining with crystal violet, and counted. Cell cycle analysis was performed with HMEM containing 0.5% "spent" media for an additional 7 days. WISH cells were used without being synchronized.

For examination of IFNτ activity, cells were replated at 2.5 × 10^5 cells/well in HMEM with 10% FBS in 6 well plates. Various dilutions of OvIFNτ alone or in combination with peptides were added to achieve a final volume of 1 ml. Plates were incubated at 37°C in 5% CO_2 for 12, 15, 18, 24, or 48 hours. Cells were treated with trypsin, collected by low speed centrifugation and washed. The cell pellet was blotted dry and 250 μl of nuclear staining solution (5 mg propidium iodide, 0.3 ml NP40 and 0.1 gm sodium citrate in 100 ml distilled H_2O) was added to each tube. The tubes were incubated at room temperature. After 10 minutes, 250 μl of RNase (500 units/ml in 1.12% sodium citrate) was added per tube and incubated an additional 20 minutes. Nuclei were filtered through 44 μm mesh, and analyzed on a FACStar (Becton Dickinson, Mountain View, CA) using the DNA Star 2.0 software.

In the cellular growth assay using colony formation of both the bovine epithelial line, MDBK, and the human amniotic line, WISH, OvIFNτ inhibited both colony size and number. Ovine IFNτ was more effective than human IFNα on the human cell line; thus, it is very potent in cross-species activity. Its activity was dose-dependent, and inhibition of proliferation could be observed at concentrations as low as 1 unit/ml. Concentrations as high as 50,000 units/ml (units of antiviral activity/ml) stopped proliferation, while cell viability was not impaired.

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Cell cycle analysis by flow cytometry with propidium iodide-stained WISH cells revealed an increased proportion of cells in G2/M after 48 hours of OvIFNτ treatment. IFNτ, therefore, appears to inhibit progress of cells through S phase. Ovine IFNτ antiproliferative effects can be observed as early as 12 hours after the initiation of culture and are maintained through 6 days.

5   The results presented above demonstrate both the antiproliferative effect of IFNτ as well as its low cytotoxicity.

EXAMPLE 14
Further Antiproliferative Effects of IFNτ

The antiproliferative effects of OvIFNτ were studied for a rat cell line and a bovine cell line.

10   The rate of ³H-thymidine incorporation was used to assess the rate of cellular proliferation.

Rat (MtBr7 .c5) or bovine kidney (MDBK) cells were seeded in phenol red-free DME-F12 medium supplemented with 3% dextran-coated charcoal stripped Controlled Process Serum Replacement 2 (CPSR 2, Sigma) and 5% dextran-coated charcoal stripped fetal bovine serum (FBS). After attaching for approximately 15-18 hours, the cells were washed once with serum-free

15   DME-F12 medium. The medium was replaced with phenol red-free DME-F12 medium supplemented with 3% stripped CPSR2, 1% stripped FBS ("3/1" medium) or 3/1 medium containing OvIFNτ at various units of antiviral activity as determined in the vesicular stomatitis virus challenge assay for interferons (Example 2). Media containing a similar dilution of buffer (undiluted buffer = 10 mM Tris, 330 mM NaCl, [TS]), in which the OvIFNτ was dissolved was

20   used for controls.

Cells were pulse labeled with ³H-thymidine for 2 hours at approximately 48 hours post-treatment. The trichloroacetic acid (TCA) precipitable incorporated counts were determined by scintillation counting. Three replicates were included per treatment. Mean values for OvIFNτ treatments were compared to samples containing comparable dilutions of carrier TS buffer.

25   Results of these experiments are shown in Table 7.

Table 7
³H-Thymidine Incorporation

Treatment	% Reduction ³H-Thymidine Incorporation
Experiment 1: MtBr7 .c5 (Rat)	
3/1	-
10³ u OvIFNτ/ml	0 (+12)

30

Treatment	% Reduction ³H-Thymidine Incorporation
1:5000 TS	-
10⁴ u OvIFNτ/ml	24
1:500 TS	-
10⁵ u OvIFNτ/ml	87
Experiment 2: MDBK	
3/1	-
10³ u OvIFNτ/ml	74
1:5000 TS	-
10⁴ u OvIFNτ/ml	83
1:500 TS	-
10⁵ u OvIFNτ/ml	83

As can be seen from Table 7, OvIFNτ drastically reduced the rate of cellular proliferation (based on thymidine incorporation) for each of the cell lines tested.

EXAMPLE 15
Antiproliferative Effects of IFNτ on Human Tumor Cell Lines

The antiproliferative activity of OvIFNτ on human tumor cell lines was evaluated by measuring the rate of ³H-thymidine incorporation into cells which have been treated with OvIFNτ.

For experiments on tumor lines that grow in suspension, 1 ml of cells were plated at from 2.5 - 5 × 10⁵ cells/well in 24-well plates. Triplicate wells received either the appropriate media, 100, 1,000 or 10,000 units/ml of OvIFNτ or equivalent antiviral concentrations of rHuIFNα2A (Lee Biomolecular). After 48 hours of incubation, cells were counted and viability assessed by trypan blue exclusion.

Adherent tumor lines were plated at 2.5 × 10⁵ cells/well in 1 ml in 6-well plates. They received interferon treatments as just described, but were trypsinized prior to counting.

Significant differences between treatments were assessed by an analysis of variance followed by Scheffe's F-test. Cell cycle analysis was performed by flow cytometry using propidium iodide.

A. Breast Adenocarcinoma Cells.

Human MCF7 breast adenocarcinoma cells were seeded from logarithmically growing cultures in phenol red-free DME-F12 medium supplemented with 3% dextran-coated charcoal

stripped CPSR and 5% dextran-coated FBS. After attaching for approximately 15-18 hours, the cells were washed once with serum-free DME-F12 medium. The medium was replaced with phenol red-free DME-F12 medium supplemented with 3% stripped CPSR2, 1% stripped FBS ("3/1" medium) or 3/1 medium containing OvIFNτ at the indicated number of units of antiviral

5   activity as determined in the vesicular stomatitis virus challenge assay for interferons. Media containing a similar dilution of buffer (undiluted buffer = 10 mM Tris, 330 mM NaCl [TS]) was used for controls. Cells were pulse labeled with ^{3}H-thymidine for 2 hours at approximately 48 hours post-treatment.

The trichloroacetic acid (TCA) precipitable incorporated counts were determined by

10   scintillation counting. Three replicates were included per treatment. Mean values for OvIFNτ treatments were compared to samples containing comparable dilutions of carrier TS buffer. The results of these analyses are shown in Table 8.

Table 8
^{3}H-Thymidine Incorporation

Treatment	% Reduction ^{3}H-Thymidine Incorporation
MCF7 Human	
3/1	-
10^3 u OvIFNτ/ml	35
1:5000 TS	-
10^4 u OvIFNτ/ml	53
1:500 TS	-
10^5 u OvIFNτ/ml	70

As can be seen from the results shown in Table 8, OvIFNτ was able to substantially reduce the rate of ^{3}H-thymidine incorporation in the human carcinoma cell line. This demonstrates the

25   efficacy of OvIFNτ in inhibiting tumor cell proliferation, in particular, mammary tumor cell proliferation.

B.   Human Promyelocytic Leukemia.

A comparison of the antiproliferative effects of OvIFNτ and IFNα was conducted using HL-60 (human leukemia) cells (Foa, et al., 1982; Todd, et al., 1981) essentially as described above

30   for MDBK cells. Both OvIFNτ and rHuIFNα inhibit HL-60 cell proliferation. Results of one of three replicate experiments are presented as mean % growth reduction ± SD in Figure 4. Figure

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4 shows that both OvIFNτ and IFNα were able to drastically reduce growth of HL-60 cells. The growth reduction for each compound exceeded 60% for each concentration tested. At 10,000 units/ml, OvIFNτ caused an approximately 80% reduction in growth while IFNα caused a 100% reduction in growth.

5   However, the data presented in Figure 4 reveal, that a substantial factor in the ability of IFNα to reduce growth was its toxic effect on the cells. At 10,000 units/ml, the toxicity of IFNα resulted in less than 25% of the cells remaining viable. By contrast, nearly 100% of the cells remained viable when OvIFNτ was applied at 10,000 units/ml.

         Figure 5 presents data demonstrating that rHuIFNα is cytotoxic. In the figure, results of one
10   of three replicate experiments are presented as mean % viability ± SD.

         C. Human Cutaneous T Cell Lymphoma.

         The cutaneous T cell lymphoma, HUT 78, responded similarly to HL-60 when treated with IFNτ (Figure 9). Both OvIFNτ and rHuIFNα reduce HUT 78 cell growth, but 10,000 units/ml of rHuIFNα decreased the cell number below that originally plated (5X10^5). This is indicative of
15   a reduction in cell viability to approximately 60%.

         Cell cycle analysis (performed by cell flow cytometry) revealed an increased proportion of cells in G2/M phase of the cell cycle upon 48 hours of treatment with both interferons (Table 9). In Table 9 the results from one of three replicate experiments are presented as the percentage of cells in each phase of the cell cycle. 10,000 events were analyzed per sample.

20   This result is likely due to the slower progress of cells through the cell cycle. In the sample treated with 10,000 units/ml of rHuIFNα, a large percentage of events with low forward and high side scatter, identifying dead cells, were present. This is consistent with the data obtained from proliferation experiments, where only OvIFNτ inhibited HUT 78 proliferation without toxicity.

Table 9
25   HUT 78 Cell Cycle Analysis

Treatment (units/ml)	G0/G1	S	G2/M
Media	44.43	49.95	5.61
100 OvIFNτ	44.35	47.45	8.20
100 rHuIFNα	40.01	57.53	2.45
1,000 OvIFNτ	41.29	50.50	8.21
1,000 rHuIFNα	41.73	44.91	13.36
10,000 OvIFNτ	42.79	42.61	14.60

10,000 rHuIFNα	18.01	71.31	10.67 (cell death)

D. Human T Cell Lymphoma.

The T cell lymphoma cell line H9 was slightly less sensitive to the antiproliferative effects of the IFNs than the tumor cell lines described above. Results of one of three replicate experiments are presented in Figure 10 as mean % growth reduction ± SD. While rHuIFNα was not toxic to the H9 cells, it failed to inhibit cell division significantly at any of the concentrations examined. In contrast, OvIFNτ was observed to reduce H9 growth by approximately 60% (Figure 10). Thus, only OvIFNτ is an effective growth inhibitor of this T cell lymphoma.

The results presented above demonstrate both the antiproliferative effect of IFNτ as well as its low cytotoxicity.

EXAMPLE 16
Preliminary *In Vivo* Treatment with OvIFNτ

Three groups of 4 C57Bl/6 mice per group were given 2.5×10^4 B16-F10 cells via the tail vein: B16-F10 is a syngeneic mouse transplantable tumor selected because of its high incidence of pulmonary metastases (Poste, *et al.*, 1981). Interferon treatment was initiated 3 days after the introduction of the tumor cells. Each mouse received 100 μl of either PBS alone, PBS containing 1×10^5 units of OvIFNτ, or PBS containing 1×10^5 units of recombinant murine IFNα (MuIFNα), i.v. per day for 3 consecutive days.

Mice were sacrificed at 21 days and the lungs were preserved in 10% buffered formalin. The frequency of pulmonary metastases were compared between control mice (PBS), OvIFNτ-treated mice, and MuIFNα-treated mice. The results of these *in vivo* administrations demonstrated that OvIFNτ dramatically reduced B16-F10 pulmonary tumors. These results support the use of IFNτ as an efficacious antineoplastic agent *in vivo*.

EXAMPLE 17
Competitive Binding of IFNτ Peptide Fragments

A. The Ability of IFNτ-Based Peptides to Block IFNτ and IFN-α Antiviral Activity.

Overlapping synthetic peptides were synthesized corresponding to the entire IFNτ sequence (Figure 6). Average hydropathicity values were calculated by taking the sum of the hydropathy values for each amino acid divided by the total number of amino acids in each sequence. Hydropathy values were taken from Kyte, *et al.* (1982).

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These peptides were of approximately the same molecular weight but differed slightly in overall hydrophilicity. Despite this difference, all peptides were antigenic as demonstrated by the production of rabbit antisera with titers greater than 1:3,000 as assessed by ELISA (Harlow, *et al.*, 1988).

5   The peptides were used to inhibit the antiviral activity (Example 2) of OvIFNτ and rBoIFNα. The results of this analysis are presented in Figure 12: 1 mM N- and C-terminal peptides both effectively blocked the antiviral activity of OvIFNτ using MDBK cells. A third peptide, representing amino acids 62-92, also reduced IFNτ antiviral activity (70% inhibition). The peptide OvIFNτ (119-150) showed minimal inhibitory activity. The OvIFNτ (34-64) and (90-122) peptides

10   had no apparent inhibitory activity.

Peptide inhibition of OvIFNτ antiviral activity was also examined as follows. Monolayers of Madin Darby bovine kidney cells were incubated with 40 units/ml OvIFNτ in the presence or absence of various concentrations of OvIFNτ peptides (see Figure 13). Results in Figure 13 are expressed as the percent of control antiviral activity: that is, in the absence of any competing

15   peptide. Data presented are the means of 6 replicate experiments. The data demonstrate that inhibition by OvIFNτ (1-37), (62-92), (119-150), and (139-172) were significantly different than OvIFNτ (34-64) and (90-122) at 10^{-3} M and 3×10^{-3} M. OvIFNτ (139-172) was significantly different than all other peptides at 10^{-3} M. Significance was assessed by analysis of variance followed by Scheffe's F test at $p < 0.05$. Thus, OvIFNτ (1-37) (62-92), (119-150), and (139-172),

20   in particular (139-172), may represent receptor binding regions for IFNτ.

The ability of the OvIFNτ peptides to inhibit bovine IFNα (BoIFNα) antiviral activity was examined as follows. Monolayers of Madin Darby bovine kidney cells were incubated with 40 units/ml bovine IFNα in the presence or absence of various concentrations of OvIFNτ peptides. The results are presented in Figure 14 and are expressed as the percent of control antiviral activity

25   in the absence of OvIFNτ peptides. The data presented are the means of 4 replicate experiments. The results indicate that inhibition by OvIFNτ (62-92), (119-150), and (139-172) were significantly different from OvIFNτ (1-37), (34-64) and (90-122) at 10^{-3} M. OvIFNτ (139-172) was significantly different than OvIFNτ (1-37), (34-64) and (90-122) at 3×10^{-3} M. Significance was assessed by analysis of variance followed by Scheffe's F test at $p < 0.05$. Thus, OvIFNτ (62-92),

30   (119-150), and (139-172), in particular (139-172), may represent common receptor binding regions for IFNτ and bovine IFNα.

Peptide inhibition by OvIFNτ peptides of human IFNα antiviral activity was also examined. Monolayers of Madin Darby bovine kidney cells were incubated with 40 units/ml human IFNα in the presence or absence of various concentrations of OvIFNτ peptides. The results are

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expressed as the percent of control antiviral activity in the absence of OvIFNτ peptides. The data
are presented in Figure 15 and are the means of 3 replicate experiments. OvIFNτ (139-172) was
significantly different from all other peptides at 10^3 M. Significance was assessed by analysis of
variance followed by Scheffe's F test at $p < 0.05$. Thus, OvIFNτ (139-172) may represent a
5   common receptor binding region for IFNτ and various IFNα(s).

The OvIFNτ peptides described above appear to have no effect on the antiviral activity of
IFNγ. Peptide inhibition of bovine IFNγ antiviral activity was evaluated as follows. Monolayers
of Madin Darby bovine kidney cells were incubated with 40 units/ml bovine IFN gamma in the
presence or absence of various concentrations of OvIFNτ peptides. Results are expressed as the
10   percent of control antiviral activity in the absence of OvIFNτ peptides. The data are presented
in Figure 16 and are the means of 3 replicate experiments. There were no significant differences
among peptides as assessed by analysis of variance followed by Scheffe's F test at $p < 0.05$.

The two synthetic peptides OvIFNτ(1-37) and OvIFNτ(139-172) also blocked OvIFNτ anti-
FIV and anti-HIV activity. Reverse transcriptase (RT) activity (Examples 12 and 13) was
15   monitored over a 14 day period in FIV-infected FET-1 cells (1×10^6/ml) and HIV-infected HPBL
(1×10^6/ml). Control cultures received no OvIFNτ. OvIFNτ was used at 100 ng/ml, and
peptides were used at 200 μM. Data from a representative experiment are expressed as cpm/ml
culture supernatant and are presented for FIV infected cells, Figure 11A, and HIV infected cells,
Figure 11B. Both the N- and C-terminus of OvIFNτ appear to be involved in its anti-retroviral
20   activity. While both peptides blocked FIV RT activity, only the C-terminal peptide, OvIFNτ(139-
172), was an efficient inhibitor of vesicular stomatitis virus activity on the feline cell line, Fc9.
Thus the C-terminal regions of type I IFNs may bind to common site on the type I IFN receptor,
while the N-terminal region may be involved in the elicitation of unique functions.

B. Anti-Peptide Sera.

25   The ability of anti-peptide antisera to inhibit OvIFNτ antiviral activity was also determined.
Antipeptide antisera inhibition of OvIFNτ antiviral activity was evaluated as follows. Monolayers
of MDBK cells were incubated with 20 units/ml of OvIFNτ in the presence a 1:30 dilution of
either preimmune sera or antisera to each of the OvIFNτ peptides described above. In Figure 17
the data from duplicate experiments are presented as the mean percent inhibition of OvIFNτ
30   antiviral activity produced by antipeptide antisera relative to the appropriate preimmune sera ±
standard error. Significant differences were assessed by analysis of variance followed by Scheffe's
F test at $p < 0.05$. Consistent with peptide inhibition of antiviral activities, sera containing
antibodies immunoreactive to OvIFNτ (1-37), OvIFNτ (62-92), and OvIFNτ (139-172) were also

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the most effective inhibitors of OvIFNτ antiviral activity, with antibodies directed against the N-terminal and C-terminal peptides being the most efficacious.

The same sera were also used to examine their effect on the binding of IFNτ to its receptor.

The IFNτ binding assay was carried out as follows. Five μg of IFNτ was iodinated for 2
5 minutes with 500 μCi of Na^{125}I (15 mCi/μg; Amersham Corporation, Arlington Heights, IL) in 25 μl of 0.5 M potassium phosphate buffer, pH 7.4, and 10 μl of chloramine-T (5 mg/ml) (Griggs, et al., 1992). The specific activity of the iodinated protein was 137 μCi/μg. For binding assays, monolayers of MDBK cells were fixed with paraformaldehyde and blocked with 5% nonfat dry milk. Cells were incubated with 5 nM ^{125}I-IFNτ in phosphate buffered saline with 1% BSA
10 for 2 hours at 4°C in the presence or absence of a 1:30 dilution of sera containing antibodies raised against IFNτ peptides or the appropriate preimmune sera. Specific binding was assessed by incubation with a 100-fold molar excess of unlabeled IFNτ. Specific binding of 36% was determined by competition with 500 nM unlabeled IFNτ. For example, total counts bound were 6850 ± 133, and a 100-fold molar excess of OvIFNτ produced 4398 ± 158 counts per minute.
15 After incubation, the monolayers were washed three times, solubilized with 1% sodium dodecyl sulfate, and the radioactivity counted. Data from three replicate experiments are presented in Figure 18 as the mean percent reduction of OvIFNτ specific binding produced by antipeptide antisera relative to the appropriate preimmune sera ± standard deviation. Significant differences were assessed by analysis of variance followed by Scheffe's F test.

20 The same sera (containing antibodies immunoreactive to OvIFNτ (1-37), OvIFNτ (62-92), and OvIFNτ (139-172)) were the most effective inhibitors of ^{125}I-IFNτ binding to its receptor on MDBK cells. The lack of effect of sera immunoreactive with other IFNτ-derived peptides was not a function of titer against OvIFNτ, since each sera had equal or greater titer to their respective peptide relative to the three inhibiting sera: similar results were obtained when sera reactivity
25 against the whole OvIFNτ molecule was assessed by ELISA for each sera.

These peptides, although apparently binding to the interferon receptor, did not in and of themselves elicit interferon-like effects in the cells.

C. Anti-Proliferative Activity.

Functionally important sites for the antiproliferative activity of IFNτ were also examined
30 using synthetic peptides (Table 10). Cellular proliferation was assayed as described above using MDBK cells. MDBK cells were cultured at 5 × 10^5 cells/well in experiments 1 and 2 or 10 × 10^5 cells in experiment 3 and treated with medium alone, IFNτ at a concentration of 300 units/ml and peptides at 1 mM for 48 hours. Duplicate wells were counted in each of three replicate experiments. For statistical analysis, data were normalized based on medium alone and assessed

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by analysis of variance followed by Least Significant Difference multiplate comparison test (p > 0.05).

Table 10
Peptide Inhibition of IFNτ Antiproliferative Activity

Treatment	Experiment 1		Experiment 2		Experiment 3	
	Cell Count	Via-bility	Cell Count	Via-bility	Cell Count	Via-bility
Medium alone	9.8×10^5	99%	13.0×10^5	96%	27.3×10^5	97%
IFNτ	5.0×10^5	98%	5.6×10^5	97%	8.3×10^5	97%
IFNτ+IFNτ(1-37)	6.3×10^5	100%	10.6×10^5	98%	13.4×10^5	100%
IFNτ+IFNτ(34-64)	5.3×10^5	96%	6.9×10^5	95%	16.0×10^5	98%
IFNτ+IFNτ(62-92)	6.5×10^5	97%	9.2×10^5	93%	8.9×10^5	96%
IFNτ+IFNτ(90-122)	5.9×10^5	100%	11.0×10^5	97%	19.6×10^5	98%
IFNτ+IFNτ(119-150)	8.4×10^5	100%	13.2×10^5	96%	31.8×10^5	90%
IFNτ+IFNτ(139-172)	5.1×10^5	100%	12.7×10^5	98%	18.9×10^5	98%

When proliferation of MDBK cells was monitored over a two-day period, cell number increased roughly 2-fold with greater than 95% viability. Addition of 300 units/ml of OvIFNτ entirely eliminated cell proliferation without a decrease in cell viability. Ovine IFNτ (119-150) was the most effective inhibitor of IFNτ antiproliferative activity.

Antisera to IFNτ (119-150), which inhibited binding of OvIFNτ to receptor, also reversed the OvIFNτ antiproliferative effect. Several other peptides, notably IFNτ (139-172), reversed the OvIFNτ antiproliferative effect, but to a lesser extent.

EXAMPLE 18
Differential Recognition of the Type I Interferon Receptor by IFN-τ and IFN-α

A. Relative Cytotoxicities of OvIFNτ and Human IFN-αA Assayed on MDBK Cells.

Cytotoxicity assays were performed MDBK cells grown to confluence in 96-well plates. Test cells were treated with various concentrations (indicated in the Figures) of IFNs, in triplicate, in 100 μl of EMEM supplemented with 2% newborn calf serum and control cells were treated with medium alone. OvIFNτ DNA coding sequences were cloned into the pHIL-S1 *Pichia* expression plasmid (Invitrogen, San Diego, CA), the plasmid was cut with *Bgl*II, and the linearized plasmid was used to transform *Pichia pastoris* (strain GS115; Invitrogen) spheroplasts according to the manufacturer's instructions. Recombinant OvIFNτ protein was expressed by transformed His⁺ Mut⁻ yeast cells and purified from the culture medium by ion-exchange and hydroxyapatite

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chromatography to homogeneity (0.8×10^8 units/mg). Purified recombinant human IFNαA (2×10^8 units/mg) was obtained from Biosource International (Camarillo, CA).

Cells were incubated at 37°C until significant cell death was evident by microscopic examination. Cells were then stained with crystal violet, the plates were washed and air-dried and

5   the dye was extracted with 2-methoxyethanol (methyl cellosolve). Absorbance of the eluted dye was measured at 570 nm.

The results are shown in Figures 22A and 22B. IFNτ was at least 30-fold less toxic to MDBK cells than IFNα. The concentration causing 50% cell death was about 2500 units/ml for IFNα in comparison to about 85,000 units/ml for IFNτ. These results further confirm that IFNτ

10   and IFNα differ markedly in their cytotoxic effects.

B.   OvIFNτ Does Not Inhibit IFN-αA-Mediated Cytotoxicity.

The potential of subtoxic concentrations of IFNτ to act as a competitive antagonist to the cytotoxic effect of IFNα was investigated using the cytotoxicity assay described immediately above.

15   In the first experiment, shown in Fig. 23A, either IFNτ (50,000 units/ml) and/or IFNαA (5,000 units/ml) were added to cells, and the assay was conducted as described immediately above. The data indicate that IFNτ treatment, at a 10-fold higher (but subtoxic) concentration than IFNαA, did not block the toxicity of IFNαA on MDBK cells.

In the second experiment, shown in Fig. 23B, the cells were treated for 1 hr at 37°C with

20   IFNτ (25,000 units/ ml) before the addition of IFNαA (5,000 units/ml) without removal of IFNτ. The results show that addition of IFNτ at a 5-fold higher concentration 1 hr prior to the addition of IFNαA to the cells did not block the toxicity of IFNαA.

Taken together, these results show that IFNτ is a poor antagonist to the cytotoxic effect of IFNαA. This was a surprising observation in view of the established structural and functional

25   homology of these two type I interferons (Jarpe, et al., 1994) and their similar specific antiviral activities on MDBK cells (Pontzer, et al., 1988). The inability of IFNτ to block the cytotoxicity of IFNαA suggests that these IFNs bind differently to the type I receptor complex, perhaps initiating different signaling events.

C.   Binding of ^{125}I-labeled IFNτ and IFNα to MDBK Cells.

30   IFNαA and IFNτ were labeled with the Bolton-Hunter reagent (mono [^{125}I]iodo derivative, ~2000 Ci/mmol, Amersham; 1 Ci = 37 GBq) as described (Langer and Pestka, 1986). Specific activity of labeled proteins was 40-70 µCi/µg. The labeled IFNs retained complete antiviral activity on MDBK cells which was unchanged for at least 4 weeks at 4°C.

Binding assays using MDBK cells were performed as described for IFNα (Zoon, et al. 1986). Confluent monolayers of MDBK cells in six-well plates precooled to 4°C were incubated with the appropriate concentrations of labeled or unlabeled IFNs in 2 ml of complete growth medium at 4°C for 4 hr for ^{125}I-IFNαA or overnight (≥ 17 hr) for ^{125}I-IFNτ to allow for binding to attain

5   equilibrium. Saturation binding data were analyzed with the "EBDA" program (McPherson 1985).

The results are shown in Figs. 24A (^{125}I-IFNτ) and 24B (^{125}I-IFNαA). Specific binding was calculated by subtracting nonspecific binding determined at each concentration in the presence of a 100-fold excess of the corresponding unlabeled IFN. Nonspecific binding was <20% for IFNτ and <7% for IFNαA. Values are plotted as mean $\pm$ SE. The insets in each figure show

10   Scatchard plots of the binding data (B, bound; F, free).

The data show that ^{125}I-IFNτ bound to MDBK cells with high specificity (Fig. 24A). Scatchard analysis of the binding (inset in Fig. 24A) revealed an apparent K_d of 3.90×10^{-10} M. The K_d value is within the range (10^{-11} to 10^{-9} M) for the binding of the various IFNαs to a variety of cell types (Rubinstein, et al., 1986; Aguet, et al., 1983) and is similar to the value for binding

15   of recombinant bovine IFNαD (3.5×10^{-10}M) and recombinant bovine IFNτ (3.7×10^{-10}M) to bovine endometrial membranes (Li and Roberts, 1994). However, Scatchard analysis of the binding of ^{125}I-IFNαA to MDBK cells (Fig. 24B) yielded an apparent K_d of 4.45×10^{-11}M for IFNαA, similar to that ($6.0 \times 10 \times 10^{-11}$M) previously reported (Zoon, et al. 1986). The total receptor concentration for ^{125}I-IFNαA (4.62 pM) was very similar to that for ^{125}I-IFNτ (4.22 pM)

20   from the Scatchard plots. These results indicate that IFNαA had nearly a 10-fold lower K_d for IFN receptors on MDBK cells than IFNτ. Further, the results suggest that this substantial difference in binding affinities is responsible for the inability of IFNτ to "compete" for receptor and block the toxicity of IFNαA.

D. Competitive Binding of ^{125}I-labeled IFNτ and IFNα to MDBK Cells.

25   Competitive binding experiments were conducted to confirm that IFNτ and IFNαA were binding to the same receptor. Confluent monolayers of MDBK cells in six-well plates were incubated, in triplicate, with the indicated concentrations of unlabeled IFNαA or IFNτ and 0.2 nM ^{125}I-IFNτ (Fig. 25A) or 0.02 nM ^{125}I-IFNαA (Fig. 25B). Values are presented as percent specific binding of control determined in the absence of competitor. A 100% value represents a mean

30   specific binding of 1673 ± 51 cpm (mean $\pm$ SE) for ^{125}I-IFNτ and 1255.7 ± 16.3 cpm (mean $\pm$ SE) for ^{125}I-IFNαA. Nonspecific binding was determined in the presence of 200 nM unlabeled IFNτ for ^{125}I-IFNτ (11% nonspecific binding) and 20 nM unlabeled IFNαA for FNaA (5% nonspecific binding) and was subtracted from total binding.

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IFNαA was a potent competitor of ^{125}I-IFNτ binding to MDBK cells (Fig. 25A). In fact, IFNαA was 40-fold more effective than IFNτ itself in inhibiting the binding of ^{125}I-IFNτ at the 50% level. Similar results were obtained when recombinant human IFNαD was used as the competitor. Cross-competition studies using ^{125}I-IFNαA (Fig. 25B) again showed that IFNαA was

5   >40-fold more effective than IFNτ in displacing ^{125}I-IFNαA at the 50% level.

These data indicate that IFNαA has a much higher affinity than IFNτ for the IFN receptors on MDBK cells, a result consistent with the 10-fold lower K_d. Moreover, the results of the competition experiments provide an explanation for the inability of excess (5:1) IFNτ to block the cytotoxic effects of IFNαA on MDBK cells. The higher receptor affinity of IFNαA suggests

10 differential receptor recognition by IFNτ and IFNαA, and explains the inability of IFNτ to block the toxicity of IFNαA.

E.   Inhibition of Binding of IFNs to MDBK Cells by Antisera Against the N- or C- termini of IFNτ.

Rabbit antisera raised against IFNτ peptides IFNτ (1-37) (N-terminal; SEQ ID NO:5) and

15 IFNτ (139-172) (C-terminal; SEQ ID NO:10) as described in Example 17 were tested for their ability to block the binding of IFNα and IFNτ to MDBK cells. Confluent monolayers of MDBK cells in 96-well plates were incubated with 0.3 μM biotinylated IFNα or IFNτ in phosphate–buffered saline (PBS) containing 5% fetal bovine serum and a 1:30 dilution of the appropriate antiserum for 3 hr at room temperature. After washing with phosphate-buffered saline containing

20 5% fetal bovine serum, plates were developed with an "EXTRAVIDIN"-alkaline phosphate conjugate using p-nitrophenyl phosphate as substrate (Sigma Chemical Co., St. Louis, MO). IFNα and IFNτ were biotinylated with the Immunopure NHS-LC-biotinylation kit (Pierce) according to the manufacturer's instructions. Specific binding was determined as described above for ^{125}I-labeled IFNs.

25 The results are shown in Figure 26. The solid bars represent inhibition of binding of IFNτ and IFNαA to MDBK cells by antisera raised against the N-terminus, while hatched bars represent inhibition by antisera raised against the C-terminus. Data are presented as mean absorbance ± SD. Control samples were treated with preimmune serum (open bars).

The data indicate that the antiserum to the N-terminal peptide IFNτ (1-37) (SEQ ID NO:5)

30 specifically blocked the binding of IFNτ, but not that of IFNα, to MDBK cells. In contrast, antiserum to the C-terminal peptide IFNτ (139-172) (SEQ ID NO:10) blocked binding of both IFNτ and IFNα. These findings are consistent with the hypothesis that IFNτ (1-37) contains an epitope that is unique to IFNτ and is instrumental in both binding and biological activity of IFNτ on MDBK cells. Furthermore, the data suggest that this differential interaction at the N termini

of IFNτ and IFNα accounts at least in part for the differential competition at the receptor on MDBK cells.

F.   Dose-response/occupancy curves for IFNτ and IFNαA on MDBK cells.

Dose-response/occupancy curves, or concentration effect curves, for (i) percent maximal antiviral activity, (ii) percent maximal cytotoxicity, and (iii) percent maximal binding for IFNτ and IFNαA on MDBK cells were calculated using the data described above and plotted as functions of concentration in Figs. 27A (IFNτ) and 27B (IFNαA). Antiviral activity was quantitated by a cytopathic-effect-inhibition assay using vesicular stomatitis virus (Armstrong 1981) as described in Example 2, and normalized using a value of 2×10^8 units/mg for IFNαA. Data from cytotoxicity dose-response curves and saturation binding curves were replotted as percent maximal values. Concentrations were determined from antiviral units, using a specific activity of 2.0×10^8 units/mg for IFNaA and 0.8×10^8 units/mg for IFNτ on MDBK cells. The symbols are as follows: percent maximal antiviral activity ($\bigcirc$), percent maximal cytotoxicity ($\square$), and percent maximal binding ($\diamondsuit$).

The results demonstrate that cytotoxicity is associated with saturation binding to the receptor, whereas antiviral activity involves fractional occupancy of the receptors. In other words, toxicity is correlated with the K_d. IFNαA binds to MDBK cells with 10-fold greater affinity than IFNτ and is toxic at much lower concentrations. The specific antiviral activities of the two IFNs are similar, 2×10^8 units/mg of protein for IFNαA and 0.8×10^8 units/mg for IFNτ, which is consistent with the induction of maximal antiviral activity with only a low fractional occupancy of receptors.

G.   Tyk2, Stat1α and Stat2 phosphorylation induced by IFNτ and IFNαA.

Binding of IFNs to receptors is translated within the cell by signal transduction machinery involving a set of tyrosine kinases and transcription factors that are activated by phosphorylation. Experiments were thus performed tp determine whether the differences in binding of IFNα and IFNτ are manifest in changes in activation of the type I receptor-associated tyrosine kinase Tyk2 and the transcription factors Stat1α and Stat2.

Daudi cells in RPMI 1640 medium (4-5 $\times$ 10^7 cells per 1-ml sample) were stimulated with IFNαA or IFNτ at 5000 units/ml or were left untreated for 4 min (Tyk2) or 10 min (Stat1α and Stat2) at 37°C. The cells were then lysed at 4°C for 20 min in 500 μl of ice-cold lysis buffer consisting of 50 mM Tris HCl (pH 7.4), 150 mM NaCl, 2 mM EGTA, 2 mM EDTA, 50 mM NaF, 20 mM B-glyceryl phosphate, 2 mM Na_3VO_4, 0.05 mM p-nitrophenyl-p-guanidinobenzoate (from a stock solution in dimethylformamide), leupeptin (10 μg/ml), pepstatin (10 μg/ml),

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aprotinin (10 μg/ml). benzamidine (5 μg/ml). 1 mM phenylmethanesulfonyl fluoride. 10% (vol/vol) glycerol, and 1% (vol/vol) "NONIDET P-40".

After immunoprecipitation of extracts (500 μl) with 1 μg of anti-Tyk2 antibodies, or a mixture of 1 μg of anti-Statlα and 1 μg of anti-Stat2 antibodies, and immunoblotting, the blots

5   were probed (Western blot) with monoclonal anti-phosphotyrosine (anti-PY) antibody (4G10) and tyrosine phosphorylation of Tyk2. Statlα, and Stat2 was detected with ECL (Amersham). The blots were then stripped of anti-PY and reprobed either with antibodies to the Tyk2 protein or with a mixture of antibodies to Statlα and Stat2.

The results showed that IFNτ was as effective as IFNα in the phosphorylation of Tyk2. No

10   differences were seen in the levels of Tyk2 protein in unstimulated cells and cells stimulated with the IFNs. Furthermore, IFNτ and IFNα induced comparable levels of phosphorylation of both Statlα and Stat2. Again, no differences were seen in the protein levels of the Statlα and Stat2 immunoprecipitated from stimulated or unstimulated cells. Thus, IFNτ is similar to IFNα in activating these signal transduction proteins associated with the type I receptor, in keeping with

15   its structural and functional similarities with the IFNαs.

EXAMPLE 19
Further Analysis of the Cellular and Anti-Viral Effects of IFNτ

A.   HIV Anti-Viral Effects.

The antiviral effects of IFNτ against HIV were evaluated by treating human PBMC cells with

20   various amounts of either recombinant ovine IFNτ (r-OvIFNτ) or recombinant human IFNα2a at the time of infection with HIV. IFNτ was present throughout the experiment. At day 7 and day 14, p24 production was determined (by ELISA (Wang, *et al.*, 1988, 1989) and compared to a zero drug control. The results of this analysis are presented in Table 11.

TABLE 11

Amounts of Drug Units/ml		% Inhibition Day 7	% Inhibition Day 14
IFNα2a	IFNτ		
10		58%, 48%	91%, 91%
	26	48%, 45%	88%, 59%
100		68%, 74%	94%, 91%
	260	58%, 51%	82%, 70%
1,000		89%, 86%	97%, 93%
	2,600	65%, 68%	87%, 79%

Amounts of Drug Units/ml		% Inhibition Day 7	% Inhibition Day 14
IFNα2a	IFNτ		
10,000		90%, 86%	99%, 99%
	26,000	77%, 85%	77%, 96%
	260,000	85%, 84%	96%, 86%

The data from these experiments support the conclusion that, at relatively low concentrations, IFNα2a and IFNτ are effective in reducing the replication of HIV in human lymphocytes.

B. *In vitro* Cytotoxicity Test in PBMC's

Human PBMC's were seeded at 5×10^5 cells/ml. Cells were stimulated at day 0 with 3 μg/ml PHA. Cells were treated with recombinant human IFNα2A (at concentrations of 10, 100, 1,000 and 10,000 units/ml) and IFNτ (at concentrations of 2.6, 26, 260, 2,600, 26,000, 260,000, and 2,600,000 units/ml) in 200 μl/wells (4 replicates of each concentration using 96 well flat bottom plates). Control cultures were given no interferons. After 4 days of incubation, cells were pulsed for 9 hours using ^{3}H-thymidine at 1 uCi/well. The cells were harvested and the incorporation of labeled thymidine into DNA was determined (Figure 8).

No cytotoxicity was observed by measuring the uptake of thymidine at any concentration of IFNτ. However, rHuIFNα2 was toxic to cells at 1,000 units/ml.

In a second experiment, the same human PBMC's were treated with either IFNτ or human IFNα2A at concentrations of 100 units/ml or 10,000 units/ml. After 3 days or 8 days of incubation, viable cells were counted by flow cytometry. The results of this analysis are presented in Table 12.

TABLE 12

Treatment (units/ml)	Number of Viable Cells × 10,000	
	Day 3	Day 8
No Treatment	735	840
IFNτ 100 units/ml	745	860
IFNτ 10,000 units/ml	695	910
IFNα2a 100 units/ml	635	750
IFNα2a 10,000 units/ml	680	495

No cytotoxicity was observed in the cells treated with IFNτ. However, there was 10% cell death in IFNα2a treated cells at Day 3 and 49% cell death at Day 8.

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C.   Inhibition of Hepatitis B Virus DNA Replication in Hepatocytes

The cell line used, HepG2-T14, is a human cell that was derived from liver cells transfected with Hepatitis B Virus (HBV). The cell line semi-stably produces HBV virus: over time the cell line's production of HBV intracellular DNA and secreted virus decreases. In order to maximize

5   production of HBV DNA and virus, the cells are pre-treated with deAZA-C (5-azacytidine; Miyoshi, *et al.*, 1992) to induce production of the virus. Treatment was for 2-3 days and the amount of induction was about a factor of two.

The cells were then treated with either the IFNα and IFNτ at levels of 0, 5,000, 10,000, 20,000 and 40,000 units per ml.

10   All levels of either IFNα or IFNτ reduced DNA production by about a factor of 2 compared to the no drug control.

D.   Inhibition of Hepatospecific Messenger RNA Production in Hepatocytes

The hepatocyte cell line HepG2-T14 (described above) was examined for the effects of IFNα and IFNτ on hepatospecific mRNA production. Cells were incubated in concentrations of IFNα

15   or IFNτ at 0, 5,000, 10,000, 20,000, and 40,000 units per ml. The messenger RNAs for the hepatocyte specific proteins Apo E and Apo A1 were detected by hybridization analysis (Sambrook, *et al.*, 1989; Maniatis, *et al.*, 1982) using probes specific for these two mRNA's (Shoulders, *et al.*, 1982; Wallis, *et al.*, 1983).

No reduction of mRNA production was seen for Apo E or Apo A1 mRNA production with

20   up to 40,000 units of either IFNα or IFNτ. This result suggests that the reduction of viral DNA replication in previous experiments was not due to the effects of IFNs on cellular house-keeping activities; rather the reduction was likely due to specific inhibition of viral replication in the host cells.

E.   *In Vitro* Toxicity of IFNβ, IFNγ and IFNτ - L929 Cell Assay

25   The toxicity of IFN treatment was measured *in vitro* using the mouse L929 cell line. L929 cells were treated with 6000 U/ml to 200,000 U/ml of either OvIFNτ or MuIFNβ. The interferons were added at time zero and the cells were incubated for 72 hours and stained with crystal violet. The percentage of living cells was determined by measuring the absorbance at 405 nm.

Exemplary data are shown in Figure 21. Values are presented as percent viability ± standard

30   error in which 100 percent is equal to the viability of L929 cells treated with media alone. At 6000 U/ml, IFNβ-treated cells exhibited a 77.0±0.6% viability. Viability of L929 cells decreased as the concentrations of IFNβ increased in a dose-dependent manner. In contrast, L929 cells

showed no decrease in viability at any of the IFNτ concentrations tested. These data indicate that, unlike IFNβ, IFNτ lacks toxicity at high concentrations *in vitro*.

Taken together, the results summarized above demonstrate that IFNτ is essentially non-toxic at concentrations at which IFNβ induces toxicity both *in vitro* and *in vivo*.

5   F.   *In Vivo Toxicity of IFNβ, IFNγ and IFNτ - Cell Counts and Weight Changes*

The effects of *in vivo* treatment with IFNτ, IFNβ and IFNα (10^5 U/injection) on total white blood cell (WBC), total lymphocyte counts and weight measurements in NZW mice were assessed as follows. Interferons (OvIFNτ, MuIFNβ, and MuIFNα) were injected intraperitoneally (i.p.) at a concentration of 10^5 U in a total volume of 0.2 ml in PBS into groups of New Zealand White

10 (NZW) mice (Jackson Laboratories, Bar Harbor, ME). Three to four animals were included in each group. White blood cell (WBC) counts were determined before injection and at selected timepoints thereafter (typically 12 and 24 hours) using a hemocytometer and standard techniques. Differential WBC counts were performed on Wright-Giemsa stained blood smears. The Before injection, the weights of the animals ranged from 20 to 23 grams.

15   The results are summarized in Table 13, below.

Table 13
In Vivo Toxicity of Interferons as Measured
by White Blood Cell Counts and Percent Weight Change

| IFN | Cell Count (Cell No. $\times 10^3$) | | | | % Lympho-cyte Depres-sion | % Weight Change 24 Hours after Injection |
| | Before Injection | | 12 hr. after Injection | | | |
	Total WBC	Lymphocytes	Total WBC	Lymphocytes		
none	7.3 ± 1.0	6.4 ± 0.7	8.0 ± 0.8	7.1 ± 0.7	0	+0.5 ± 0.7
τ	6.7 ± 0.7	5.9 ± 0.6	6.7 ± 0.5	5.8 ± 0.4	1.7	+1.3 ± 0.5
β	7.0 ± 1.4	6.0 ± 0.5	6.8 ± 0.8	4.1 ± 0.3	31.7	-20.0 ± 1.0
α	6.0 ± 0.8	5.2 ± 0.7	4.8 ± 0.5	2.3 ± 0.2	55.8	-8.5 ± 2.0

No significant differences in WBC counts, lymphocyte counts or weight change were
25 observed between IFNτ-treated and untreated mice. In contrast, IFNβ-treated mice exhibited a 31.7% depression in lymphocyte counts 12 hours after injection, which continued for at least the next 12 hours. IFNα-treated mice exhibited a 55.8% lymphocyte depression and significant weight loss 12 hours after injection. These data indicate that, unlike IFNβ and IFNα, IFNτ lacks toxicity *in vivo* at the above concentrations as evidenced by peripheral blood cell counts and weight
30 measurements.

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EXAMPLE 20

Isolation of Hybrid Interferon Fusion Protein

Sepharose 4B beads conjugated with anti-beta galactosidase is purchased from Promega. The beads are packed in 2 ml column and washed successively with phosphate-buffered saline with

5 0.02% sodium azide and 10 ml TX buffer (10 mM Tris buffer, pH 7.4, 1% aprotinin).

A hybrid interferon coding sequence is cloned into the polylinker site of lambda gt11. The chimeric coding sequence is placed in-frame with the amino terminal β-galactosidase coding sequences in lambda gt11. Lysogens infected with gt11/IFN are used to inoculate 500 ml of NZYDT broth. The culture is incubated at 32°C with aeration to an O.D. of about 0.2 to 0.4,

10 then brought to 43°C quickly in a 43°C water bath for 15 minutes to induce gt11 peptide synthesis, and incubated further at 37°C for 1 hour. The cells are pelleted by centrifugation, suspended in 10 ml of lysis buffer (10 mM Tris, pH 7.4 containing 2% "TRITON X-100" and 1% aprotinin added just before use.

The resuspended cells are frozen in liquid nitrogen then thawed, resulting in substantially

15 complete cell lysis. The lysate is treated with DNaseI to digest bacterial and phage DNA, as evidenced by a gradual loss of viscosity in the lysate. Non-solubilized material is removed by centrifugation.

The clarified lysate material is loaded on the Sepharose column, the ends of the column closed, and the column placed on a rotary shaker for 2 hrs. at room temperature and 16 hours at

20 4°C. After the column settles, it is washed with 10 ml of TX buffer. The fused protein is eluted with 0.1 M carbonate/bicarbonate buffer, pH10. Typically, 14 ml of the elution buffer is passed through the column, and the fusion protein is eluted in the first 4-6 ml of eluate.

The eluate containing the fusion protein is concentrated in "CENTRICON-30" cartridges (Amicon, Danvers, Mass.). The final protein concentrate is resuspended in, for example, 400 μl

25 PBS buffer. Protein purity is analyzed by SDS-PAGE.

For polyclonal antibodies, the purified fused protein is injected subcutaneously in Freund's adjuvant in a rabbit. Approximately 1 mg of fused protein is injected at days 0 and 21, and rabbit serum is typically collected at 6 and 8 weeks.

EXAMPLE 21

30

Preparation of Anti-hybIFN Antibody

A.   Expression of Glutathione-S-Transferase Fusion Proteins.

A hybrid interferon coding sequence chimeric nuleic acid molecule is cloned into the pGEX vector (Boyer, et al., 1992; Frangioni, et al., 1993; Guan, et al., 1991; Hakes, et al., 1992;

Smith, D.B., *et al.*, 1988). The pGEX vector (Smith, D.B., *et al.*, 1988) was modified by insertion of a thrombin cleavage sequence in-frame with the glutathione-S-transferase protein (GST − sj26 coding sequence). This vector is designated pGEXthr. The hybrid IFN (hybIFN) coding sequence is placed in-frame with the sj26-thrombin coding sequences (Guan, *et al.*, 1991; Hakes, 5   *et al.*, 1992).

The hybrid interferon coding sequence is ligated to the linearized pGEXthr vector. The ligation mixture is transformed into *E. coli* and ampicillin resistant colonies are selected. Plasmids are isolated from the ampicillin resistant colonies and analyzed by restriction enzyme digestion to identify clones containing the IFN insert (vector designated pGEXthr-hybIFN).

10   *E. coli* strain XL-I Blue is transformed with pGEXthr-hybIFN and is grown at 37°C overnight. DNA is prepared from randomly-picked colonies. The presence of the insert coding sequence is typically confirmed by (i) restriction digest mapping, (ii) hybridization screening using labelled hybIFN probes (*i.e.*, Southern analysis), or (iii) direct DNA sequence analysis.

B.   Partial Purification of Fusion Proteins.

15   A pGEXthr-hybIFN clone is grown overnight. The overnight culture is diluted 1:10 with LB medium containing ampicillin and grown for one hour at 37°C. Alternatively, the overnight culture is diluted 1:100 and grown to OD of 0.5-1.0 before addition of IPTG (isopropylthio-β-galactoside). IPTG (GIBCO-BRL, Gaithersburg MD) is added to a final concentration of 0.2-0.5 mM for the induction of protein expression and the incubation is typically continued for 2-5 hours, 20   preferably 3.5 hours.

Bacterial cells are harvested by centrifugation and resuspended in 1/100 culture volume of MTPBS (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4). Cells are lysed by lysozyme, sonication or French press, and lysates cleared of cellular debris by centrifugation.

An aliquot of the supernatant obtained from IPTG-induced cultures of pGEXthr-hybIFN-25   containing cells and an aliquot of the supernatant obtained from IPTG-induced cultures of pGEXthr-vector alone are analyzed by SDS-polyacrylamide gel electrophoresis followed by Western blotting, as described below.

If necessary, the extracts can be concentrated by ultrafiltration using, for example, a "CENTRICON 10" filter.

30   Alternatively, the fusion proteins are partially purified over a glutathione agarose affinity column as described in detail by Smith, D.B., *et al.*, 1988. In this method, 100 ml cultures are grown overnight. The cultures are diluted to 1 liter, and the cells grown another hour at 37°C. Expression of the fusion proteins is induced using IPTG. The induced cultures are grown at 37°C for 3.5 hours. Cells are harvested and a sonicator used to lyse the cells. Cellular debris is

pelleted and the clear lysate loaded onto a glutathione "SEPHAROSE" column. The column is washed with several column volumes. The fusion protein is eluted from the affinity column with reduced glutathione and dialyzed. The IFN can be liberated from the combined hybrid protein by treatment with thrombin. The sj26 and hybIFN fragments of the combined hybrid protein can then

5   be separated by size fractionation over columns or on gels.

Alternatively, the hybIFN portion of the combined hybrid protein is released from the column by treatment with thrombin (Guan, *et al.*, 1991; Hakes, *et al.*, 1992).

C. Antibodies Against the Fusion Protein.

The purified Sj26/IFN fused protein is injected subcutaneously in Freund's adjuvant in a

10   rabbit. Approximately 1 mg of fused protein is injected at days 0 and 21, and rabbit serum is typically collected at 6 and 8 weeks. A second rabbit is similarly immunized with purified Sj26 protein obtained from control bacterial lysate.

Minilysates from the following bacterial cultures are prepared: (1) KM392 cells infected with pGEXthr and pGEXthr containing the hybIFN insert; and (2) cells infected with lambda gt11

15   containing the hybIFN insert. The minilysates and a commercial source β-galactosidase are fractionated by SDS-PAGE, and the bands transferred to nitrocellulose filters for Western blotting (Sambrook, *et al.*, 1989; Ausubel, *et al.*, 1988).

Summarizing the expected results, serum from control (Sj26) rabbits is immunoreactive with each of the Sj26 and Sj26 fused protein antigens. Serum from the animal immunized with

20   Sj26/hybIFN fused protein is reactive with all Sj-26 and beta-gal fusion proteins containing hybIFN coding sequences, indicating the presence of specific immunoreaction with the hybIFN antigen. None of the sera are expected to be immunoreactive with beta-galactosidase.

Anti-hybIFN antibody present in the sera from the animal immunized with the Sj26/hybIFN is purified by affinity chromatography (using immobilized recombinantly produced hybIFN as

25   ligand, essentially as described above in Example 12 for the anti-beta-galactosidase antibody).

While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications and changes may be made without departing from the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
    (i) APPLICANT: UNIVERSITY OF FLORIDA

    (ii) TITLE OF INVENTION: Hybrid Interferon Compositions and
          Methods of Use

    (iii) NUMBER OF SEQUENCES: 55

    (iv) CORRESPONDENCE ADDRESS:
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    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE: 11-APR-1997
        (C) CLASSIFICATION:

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        (A) APPLICATION NUMBER: US 08/631,328
        (B) FILING DATE: 12-APR-1996

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(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 516 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: circular

    (ii) MOLECULE TYPE: DNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Ovis aries
        (B) STRAIN: Domestic
        (D) DEVELOPMENTAL STAGE: Blastula (blastocyst)
        (F) TISSUE TYPE: Trophectoderm
        (G) CELL TYPE: Mononuclear trophectoderm cells

    (vii) IMMEDIATE SOURCE:
        (B) CLONE: oTP-1a

    (viii) POSITION IN GENOME:
        (C) UNITS: bp

```
(ix)  FEATURE:
      (A)  NAME/KEY: CDS
      (B)  LOCATION: 1..516
(x)  PUBLICATION INFORMATION:
      (A)  AUTHORS: Ott, Troy L
                    Van Heeke, Gino
                    Johnson, Howard M
                    Bazer, Fuller W
      (B)  TITLE: Cloning and Expression in Saccharomyces
                  cerevisiae of a Synthetic Gene for the Type I
                  Trophoblast Interferon Ovine Trophoblast
                  Protein-1:Purification and Antiviral Activity
      (C)  JOURNAL: J. Interferon Res.
      (D)  VOLUME: 11
      (F)  PAGES: 357-364
      (G)  DATE: 1991
      (K)  RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 516


(xi)  SEQUENCE DESCRIPTION: SEQ ID NO:1:


TGC TAC CTG TCG CGA AAA CTG ATG CTG GAC GCT CGA GAA AAT TTA AAA
48
Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys
 1               5                  10                  15


CTG CTG GAC CGT ATG AAT CGA TTG TCT CCG CAC AGC TGC CTG CAA GAC
96
Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
                20                  25                  30


CGG AAA GAC TTC GGT CTG CCG CAG GAA ATG GTT GAA GGT GAC CAA CTG      144
Arg Lys Asp Phe Gly Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu
             35                  40                  45


CAA AAA GAC CAA GCT TTC CCG GTA CTG TAT GAA ATG CTG CAG CAG TCT      192
Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser
         50                  55                  60


TTC AAC CTG TTC TAC ACT GAA CAT TCT TCG GCC GCT TGG GAC ACT ACT      240
Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
 65                  70                  75                  80


CTT CTA GAA CAA CTG TGC ACT GGT CTG CAA CAG CAA CTG GAC CAT CTG      288
Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu
                85                  90                  95


GAC ACT TGC CGT GGC CAG GTT ATG GGT GAA GAA GAC TCT GAA CTG GGT      336
Asp Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly
            100                 105                 110


AAC ATG GAT CCG ATC GTT ACT GTT AAA AAA TAT TTC CAG GGT ATC TAC      384
Asn Met Asp Pro Ile Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr
            115                 120                 125


GAC TAC CTG CAG GAA AAA GGT TAC TCT GAC TGC GCT TGG GAA ATC GTA      432
Asp Tyr Leu Gln Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
        130                 135                 140


CGC GTT GAA ATG ATG CGG GCC CTG ACT GTG TCG ACT ACT CTG CAA AAA      480
Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr Leu Gln Lys
145                 150                 155                 160


CGG TTA ACT AAA ATG GGT GGT GAC CTG AAT TCT CCG                      516
Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro
            165                 170
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(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 172 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
       (C) INDIVIDUAL ISOLATE: amino acid sequence of a mature
           OvIFNtau protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys
 1               5                  10                  15

Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
            20                  25                  30

Arg Lys Asp Phe Gly Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu
        35                  40                  45

Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser
    50                  55                  60

Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
65                  70                  75                  80

Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu
                85                  90                  95

Asp Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly
            100                 105                 110

Asn Met Asp Pro Ile Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr
            115                 120                 125

Asp Tyr Leu Gln Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
    130                 135                 140

Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr Leu Gln Lys
145                 150                 155                 160

Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro
                165                 170
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(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 516 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (vi) ORIGINAL SOURCE:
       (C) INDIVIDUAL ISOLATE: synthetic nucleotide sequence encoding
           a mature human interferon-tau protein, HuIFNtau1.

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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TGTGACTTGT CTCAAAACCA CGTTTTGGTT GGTAGAAAGA ACTTAAGACT ACTAGACGAA
60
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ATGAGACGTC TATCTCCACG CTTCTGTCTA CAAGACAGAA AGGACTTCGC TTTGCCTCAG    120

GAAATGGTTG AAGGTGGCCA ACTACAAGAA GCTCAAGCGA TATCTGTTTT GCACGAAATG    180

TTGCAACAAA GCTTCAACTT GTTCCACACC GAACACTCTT CGGCCGCTTG GGACACCACC    240

TTGTTGGAAC AGCTCAGAAC CGGTTTGCAC CAACAATTGG ACAACTTGGA TGCATGTTTG    300

GGTCAAGTTA TGGGTGAAGA AGACTCTGCT CTCGGGAGAA CCGGTCCAAC GCTAGCTTTG    360

AAGAGATACT TCCAAGGTAT CCACGTTTAC TTGAAGGAAA AGGGTTACTC TGACTGTGCT    420

TGGGAAACCG TGCGTCTAGA AATCATGCGT AGCTTCTCTT CTTTGATCAG CTTGCAAGAA    480

AGATTACGTA TGATGGACGG TGACTTGTCG AGCCCA                              516
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(2) INFORMATION FOR SEQ ID NO:4:

       (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 172 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: protein

       (vi) ORIGINAL SOURCE:
          (C) INDIVIDUAL ISOLATE: amino acid sequence for a mature
                HuIFNtau protein, HuIFNtau1.

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
1               5                   10                  15

Leu Leu Asp Glu Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp
            20                  25                  30

Arg Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu
        35                  40                  45

Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
    50                  55                  60

Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
65              70                  75                  80

Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu
            85                  90                  95

Asp Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly
            100                 105                 110

Arg Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His
            115             -       120             125

Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val
        130                 135                 140

Arg Leu Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu
145                 150                 155                 160

Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: amino acid sequence of fragment 1-37
            of SEQ ID NO:2

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys
           5   10   15

Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
         20   25   30

Arg Lys Asp Phe Gly
       35

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 31 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: amino acid sequence of fragment 34-64
            of SEQ ID NO:2

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Lys Asp Phe Gly Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu Gln
           5   10   15

Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser
         20   25   30

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 31 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: amino acid sequence of fragment 62-92
            of SEQ ID NO:2

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gln Gln Ser Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp
           5   10   15

Asp Thr Thr Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln
         20   25   30

86

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: amino acid sequence of fragment 90-122
                of SEQ ID NO:2

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gln Gln Gln Leu Asp His Leu Asp Thr Cys Arg Gly Gln Val Met Gly
           5   10   15

Glu Glu Asp Ser Glu Leu Gly Asn Met Asp Pro Ile Val Thr Val Lys
        20   25   30

Lys

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: amino acid sequence of fragment 119-150
                of SEQ ID NO:2

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr Asp Tyr Leu Gln Glu Lys
           5   10   15

Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val Arg Val Glu Met Met Arg
        20   25   30

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 34 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: amino acid sequence of fragment 139-172
                of SEQ ID NO:2

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Ala Trp Glu Ile Val Arg Val Glu Met Met Arg Ala Leu Thr Val
           5   10   15

Ser Thr Thr Leu Gln Lys Arg Leu Thr Lys Met Gly Gly Asp Leu Asn
        20   25   30

Ser Pro

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 588 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
      (C) INDIVIDUAL ISOLATE: HuIFNtau1 Human Interferon Tau coding
                  sequence with a leader sequence.

    (ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 1..585

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
ATG GCC TTC GTG CTC TCT CTA CTC ATG GCC CTG GTG CTG GTC AGC TAC        48
Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
  1               5                  10                  15

GGC CCA GGA GGA TCC CTG GGT TGT GAC CTG TCT CAG AAC CAC GTG CTG        96
Gly Pro Gly Gly Ser Leu Gly Cys Asp Leu Ser Gln Asn His Val Leu
             20                  25                  30

GTT GGC AGG AAG AAC CTC AGG CTC CTG GAC GAA ATG AGG AGA CTC TCC       144
Val Gly Arg Lys Asn Leu Arg Leu Leu Asp Glu Met Arg Arg Leu Ser
         35                  40                  45

CCT CGC TTT TGT CTG CAG GAC AGA AAA GAC TTC GCT TTA CCC CAG GAA       192
Pro Arg Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Leu Pro Gln Glu
     50                  55                  60

ATG GTG GAG GGC GGC CAG CTC CAG GAG GCC CAG GCC ATC TCT GTG CTC       240
Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu
 65                  70                  75                  80

CAT GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA GAG CAC TCC       288
His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser
                 85                  90                  95

TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC ACT GGA CTC       336
Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu
                100                 105                 110

CAT CAG CAG CTG GAC AAC CTG GAT GCC TGC CTG GGG CAG GTG ATG GGA       384
His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly
            115                 120                 125

GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG GCT CTG AAG       432
Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Leu Lys
        130                 135                 140

AGG TAC TTC CAG GGC ATC CAT GTC TAC CTG AAA GAG AAG GGA TAC AGC       480
Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser
145                 150                 155                 160
```

```
GAC TGC GCC TGG GAA ACC GTC AGA CTG GAA ATC ATG AGA TCC TTC TCT      528
Asp Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser
                165               170                 175

TCA TTA ATC AGC TTG CAA GAA AGG TTA AGA ATG ATG GAT GGA GAC CTG      576
Ser Leu Ile Ser Leu Gln Glu Arg Leu Arg Met Met Asp Gly Asp Leu
                180               185                 190

AGC TCA CCT TGA                                                      588
Ser Ser Pro
        195
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 195 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
                of SEQ ID NO:11 (HuIFNtau1).

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
 1               5                  10                  15

Gly Pro Gly Gly Ser Leu Gly Cys Asp Leu Ser Gln Asn His Val Leu
            20                  25                  30

Val Gly Arg Lys Asn Leu Arg Leu Leu Asp Glu Met Arg Arg Leu Ser
        35                  40                  45

Pro Arg Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Leu Pro Gln Glu
    50                  55                  60

Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser
                85                  90                  95

Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu
            100                 105                 110

His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly
        115                 120                 125

Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Leu Lys
    130                 135                 140

Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser
145                 150                 155                 160

Asp Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser
                165                 170                 175

Ser Leu Ile Ser Leu Gln Glu Arg Leu Arg Met Met Asp Gly Asp Leu
            180                 185                 190

Ser Ser Pro
        195
```

```
(2) INFORMATION FOR SEQ ID NO:13:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 bases
            (B) TYPE: nucleic acid
 5          (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA (synthetic)

        (vi) ORIGINAL SOURCE:
            (C) INDIVIDUAL ISOLATE: 25-mer synthetic oligonucleotide

10       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
CCTGTCTGCA GGACAGAAAA GACTT
25


    (2) INFORMATION FOR SEQ ID NO:14:

        (i) SEQUENCE CHARACTERISTICS:
15          (A) LENGTH: 25 bases
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA (synthetic)

20       (vi) ORIGINAL SOURCE:
            (C) INDIVIDUAL ISOLATE: 25-mer synthetic oligonucleotide

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
TCTGAATTCT GACGATTTCC CAGGC
25

25  (2) INFORMATION FOR SEQ ID NO:15:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 37 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

30       (ii) MOLECULE TYPE: peptide

        (iii) HYPOTHETICAL: NO

        (vi) ORIGINAL SOURCE:
            (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                                    1-37 of SEQ ID NO:4

35       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

        Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
        1               5                   10                  15

        Leu Leu Asp Glu Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp
                        20                  25                  30

40      Arg Lys Asp Phe Ala
                        35


    (2) INFORMATION FOR SEQ ID NO:16:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 31 amino acids
45          (B) TYPE: amino acid
```

```
                    (D)  TOPOLOGY: linear

              (ii)  MOLECULE TYPE: peptide

              (iii) HYPOTHETICAL: NO

              (vi)  ORIGINAL SOURCE:
                    (C)  INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                         34-64 of SEQ ID NO:4


              (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:16:

              Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu Gln
              1               5                   10                  15

              Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
                        20                  25                  30


      (2)  INFORMATION FOR SEQ ID NO:17:

              (i)   SEQUENCE CHARACTERISTICS:
                    (A)  LENGTH: 31 amino acids
                    (B)  TYPE: amino acid
                    (D)  TOPOLOGY: linear

              (ii)  MOLECULE TYPE: peptide

              (iii) HYPOTHETICAL: NO

              (vi)  ORIGINAL SOURCE:
                    (C)  INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                         62-92 of SEQ ID NO:4

              (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:17:

              Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp
              1               5                   10                  15

              Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln
                        20                  25                  30

      (2)  INFORMATION FOR SEQ ID NO:18:

              (i)   SEQUENCE CHARACTERISTICS:
                    (A)  LENGTH: 33 amino acids
                    (B)  TYPE: amino acid
                    (D)  TOPOLOGY: linear

              (ii)  MOLECULE TYPE: peptide

              (iii) HYPOTHETICAL: NO

              (vi)  ORIGINAL SOURCE:
                    (C)  INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                         90-122 of SEQ ID NO:4


              (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:18:

              His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly
              1               5                   10                  15

              Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Leu Lys
                        20                  25                  30
```

Arg

(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
           119-150 of SEQ ID NO:4

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ala Leu Lys Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys
1   5   10   15

Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg
        20   25   30

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 34 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
           139-172 of SEQ ID NO:4

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser Ser
1   5   10   15

Leu Ile Ser Leu Gln Glu Arg Leu Arg Met Met Asp Gly Asp Leu Ser
        20   25   30

Ser Pro

(2) INFORMATION FOR SEQ ID NO:21:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 299 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA to mRNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: HuIFNtau6

(ix) FEATURE:
    (A) NAME/KEY: CDS
    (B) LOCATION: 2..298

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
C CAG GAG ATG GTG GAG GGC GGC CAG CTC CAG GAG GCC CAG GCC ATC    46
  Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile
  1               5                  10                  15

TCT GTG CTC CAC AAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA   94
Ser Val Leu His Lys Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr
            20                  25                  30

GAG CGC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC   142
Glu Arg Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg
                35                  40                  45

ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG GAC GCC TGC CTG GGG CAG   190
Thr Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln
            50                  55                  60

GTG ACG GGA GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG   238
Val Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu
            65                  70                  75

GCC GTG AAG AGC TAC TTC CAG GGC ATC CAT ATC TAC CTG CAA GAG AAG   286
Ala Val Lys Ser Tyr Phe Gln Gly Ile His Ile Tyr Leu Gln Glu Lys
    80                  85                  90                  95

                                                                 299
GGA TAC AGC GAC T
Gly Tyr Ser Asp
```

(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 99 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
                         of SEQ ID NO:21 (HuIFNtau6).

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser
1               5                  10                  15

Val Leu His Lys Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu
            20                  25                  30

Arg Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr
        35                  40                  45

Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val
    50                  55                  60

Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala
65                  70                  75                  80

Val Lys Ser Tyr Phe Gln Gly Ile His Ile Tyr Leu Gln Glu Lys Gly
        85                  90                  95
```

Tyr Ser Asp

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 288 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: double
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA to mRNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
       (C) INDIVIDUAL ISOLATE: HuIFNtau7

    (ix) FEATURE:
       (A) NAME/KEY: CDS
       (B) LOCATION: 2..286

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
C CAG GAG ATG GTG GAG GTC AGC CAG TTC CAG GAG GCC CAG GCC ATT
46
  Gln Glu Met Val Glu Val Ser Gln Phe Gln Glu Ala Gln Ala Ile
   1           5                 10                  15

TCT GTG CTC CAT GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC AAA
94
Ser Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Lys
                   20                 25                  30

GAG CGC TCC TCT GCT GCC TGG GAC ACT ACC CTC CTG GAG CAG CTC CTC     142
Glu Arg Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Leu
               35                 40                  45

ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG GAT GCC TGT CTG GGG CAG     190
Thr Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln
               50                 55                  60

TTG ACT GGA GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG     238
Leu Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu
       65                 70                  75

GCC GTG AAG AGC TAC TTC CAG GGC ATC CAT GTC TAC CTG CAA GAG AAG     286
Ala Val Lys Ser Tyr Phe Gln Gly Ile His Val Tyr Leu Gln Glu Lys
   80                 85                  90                  95

GG                                                                  288
```

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 95 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
       (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence

of SEQ ID NO:23 (HuIFNtau7).

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Gln Glu Met Val Glu Val Ser Gln Phe Gln Glu Ala Gln Ala Ile Ser
 1           5                  10                  15
```

5

```
Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Lys Glu
              20                  25                  30
```

```
Arg Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Leu Thr
          35                  40                  45
```

10

```
Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Leu
          50                  55                  60
```

```
Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala
 65                  70                  75                  80
```

```
Val Lys Ser Tyr Phe Gln Gly Ile His Val Tyr Leu Gln Glu Lys
                  85                  90                  95
```

15   (2) INFORMATION FOR SEQ ID NO:25:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 307 base pairs
            (B) TYPE: nucleic acid

20   (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: cDNA to mRNA

      (iii) HYPOTHETICAL: NO

       (iv) ANTI-SENSE: NO

       (vi) ORIGINAL SOURCE:
25   (C) INDIVIDUAL ISOLATE: HuIFNtau4

       (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 2..307

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

30

```
C CAG GAG ATG GTG GAG GGT GGC CAG CTC CAG GAG GCC CAG GCC ATC
46
  Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile
   1           5                  10                  15
```

35

```
TCT GTG CTC CAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA
94
Ser Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr
              20                  25                  30
```

```
GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC    142
Glu His Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg
          35                  40                  45
```

40

```
ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG GAT GCC TGC CTG GGG CAG    190
Thr Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln
          50                  55                  60
```

```
GTG ACG GGA GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG      238
Val Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu
     65                  70                  75

GCC ATG AAG ACG TAT TTC CAG GGC ATC CAT GTC TAC CTG AAA GAG AAG      286
Ala Met Lys Thr Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys
 80                  85                  90                  95

GGA TAT AGT GAC TGC GCC TGG                                          307
Gly Tyr Ser Asp Cys Ala Trp
            100
```

(2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 102 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
                     of SEQ ID NO:25 (HuIFNtau4).

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser
 1               5               10                  15

Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu
            20                  25                  30

His Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr
            35                  40                  45

Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val
        50                  55                  60

Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala
 65                  70                  75                  80

Met Lys Thr Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly
                85                  90                  95

Tyr Ser Asp Cys Ala Trp
            100
```

(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 294 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA to mRNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: HuIFNtau5

```
        (ix)  FEATURE:
                (A)  NAME/KEY: CDS
                (B)  LOCATION: 2..292


        (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:27:

  5   C CAG GAG ATG GTG GAG GGT GGC CAG CTC CAG GAG GCC CAG GCC ATC
      46
        Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile
          1               5                   10                  15

 10   TCT GTG CTC CAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA
      94
      Ser Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr
                       20                  25                  30

 15   GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC   142
      Glu His Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg
                   35                  40                  45

 20   ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG GAT GCC TGC CTG GGG CAG   190
      Thr Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln
               50                  55                  60

      GTG ACG GGA GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG   238
      Val Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu
           65                  70                  75

      GCC ATG AAG ACG TAT TTC CAG GGC ATC CAT GTC TAC CTG AAA GAG AAG   286
      Ala Met Lys Thr Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys
       80                  85                  90                  95

 25   GGA TAT AG                                                        294
      Gly Tyr

      (2)  INFORMATION FOR SEQ ID NO:28:

 30          (i)  SEQUENCE CHARACTERISTICS:
                    (A)  LENGTH: 97 amino acids
                    (B)  TYPE: amino acid
                    (D)  TOPOLOGY: linear

            (ii)  MOLECULE TYPE: protein

            (vi)  ORIGINAL SOURCE:
 35                 (C)  INDIVIDUAL ISOLATE: predicted amino acid coding sequence
                                       of SEQ ID NO:27 (HuIFNtau5).

            (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:28:

      Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser
        1               5                   10                  15

 40   Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu
                       20                  25                  30

      His Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr
                   35                  40                  45

      Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val
               50                  55                  60

 45   Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala
           65                  70                  75                  80

      Met Lys Thr Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly
```

                        85   90   95

Tyr
(2) INFORMATION FOR SEQ ID NO:29:
        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 516 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA (genomic)

        (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

        (vi) ORIGINAL SOURCE:
                (C) INDIVIDUAL ISOLATE: HuIFNtau2

        (ix) FEATURE:
                (A) NAME/KEY: CDS
                (B) LOCATION: 1..516

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC AGG AAG AAC CTC AGG CTC
48
Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg Leu
 1   5   10   15

CTG GAC CAA ATG AGG AGA CTC TCC CCT CGC TTT TGT CTG CAG GAC AGA
96
Leu Asp Gln Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp Arg
             20   25   30

AAA GAC TTC GCT TTA CCC TAG GAA ATG GTG GAG GGC GGC CAG CTC CAG   144
Lys Asp Phe Ala Leu Pro   Glu Met Val Glu Gly Gly Gln Leu Gln
             35   40   45

GAG GCC CAG GCC ATC TCT GTG CTC CAT GAG ATG CTC CAG CAG AGC TTC   192
Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser Phe
         50   55   60

AAC CTC TTC CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC   240
Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr Leu
 65   70   75   80

CTG GAG CAG CTC CGC ACT GGA CTC CAT CAG CAG CTG GAC AAC CTG GAT   288
Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu Asp
                 85   90   95

GCC TGC CTG GGG CAG GTG ATG GGA GAG GAA GAC TCT GCC CTG GGA AGG   336
Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly Arg
                 100   105   110

ACG GGC CCC ACC CTG GCT CTG AAG AGG TAC TTC CAG GGC ATC CAT GTC   384
Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His Val
             115   120   125

TAC CTG AAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ACC GTC AGA   432
Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val Arg
         130   135   140

GTG GAA ATC ATG AGA TCC TTC TCT TCA TTA ATC AGC TTG CAA GAA AGG   480
Val Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu Arg
145   150   155   160

```
TTA AGA ATG ATG GAT GGA GAC CTG AGC TCA CCT TGA                516
Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
            165               170
```

      (ix) FEATURE:
           (A) NAME/KEY: Modified-site
           (B) LOCATION: 115-117
           (D) OTHER INFORMATION: /note= "to allow expression of the
                                         encoded protein this site can be modified
                                       to encode an amino acid, e.g., Gln"

(2) INFORMATION FOR SEQ ID NO:30:

         (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 171 amino acids
              (B) TYPE: amino acid
              (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (vi) ORIGINAL SOURCE:
             (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
                                      of SEQ ID NO:29 (HuIFNtau2).

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg Leu
 1               5               10          15

Leu Asp Gln Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp Arg
            20              25              30

Lys Asp Phe Ala Leu Pro Xaa Glu Met Val Glu Gly Gly Gln Leu Gln
            35          40              45

Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser Phe
    50              55              60

Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr Leu
    65          70          75                  80

Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu Asp
            85              90              95

Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly Arg
            100             105             110

Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His Val
            115             120             125

Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val Arg
    130             135             140

Val Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu Arg
    145             150             155             160

Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
            165             170
```

      (ix) FEATURE:
           (A) NAME/KEY: Modified-site
           (B) LOCATION: 39
           (D) OTHER INFORMATION: /note= "where Xaa a selected amino acid,
                                         for example, Gln"

(2) INFORMATION FOR SEQ ID NO:31:

```
          (i)  SEQUENCE CHARACTERISTICS:
               (A)  LENGTH: 588 base pairs
               (B)  TYPE: nucleic acid
               (C)  STRANDEDNESS: double
  5            (D)  TOPOLOGY: linear

         (ii)  MOLECULE TYPE: DNA (genomic)

        (iii)  HYPOTHETICAL: NO

         (iv)  ANTI-SENSE: NO

         (vi)  ORIGINAL SOURCE:
 10            (C)  INDIVIDUAL ISOLATE: HuIFNtau3

         (ix)  FEATURE:
               (A)  NAME/KEY: CDS
               (B)  LOCATION: 1..588


         (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:31:

 15      ATG GCC TTC GTG CTC TCT CTA CTC ATG GCC CTG GTG CTG GTC AGC TAC
         48
         Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
          1               5                  10                  15

         GGC CCG GGA GGA TCC CTG CGG TGT GAC CTG TCT CAG AAC CAC GTG CTG
 20      96
         Gly Pro Gly Gly Ser Leu Arg Cys Asp Leu Ser Gln Asn His Val Leu
                        20                  25                  30

         GTT GGC AGC CAG AAC CTC AGG CTC CTG GGC CAA ATG AGG AGA CTC TCC      144
         Val Gly Ser Gln Asn Leu Arg Leu Leu Gly Gln Met Arg Arg Leu Ser
 25                     35                  40                  45

         CTT CGC TTC TGT CTG CAG GAC AGA AAA GAC TTC GCT TTC CCC CAG GAG      192
         Leu Arg Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Phe Pro Gln Glu
                 50                  55                  60

         ATG GTG GAG GGT GGC CAG CTC CAG GAG GCC CAG GCC ATC TCT GTG CTC      240
 30      Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu
          65                  70                  75                  80

         CAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA GAG CAC TCC      288
         His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser
                             85                  90                  95

 35      TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC ACT GGA CTC      336
         Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu
                        100                 105                 110

         CAT CAG CAG CTG GAT GAC CTG GAT GCC TGC CTG GGG CAG GTG ACG GGA      384
         His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val Thr Gly
 40                     115                 120                 125

         GAG GAA GAC TCT GCC CTG GGA AGA ACG GGC CCC ACC CTG GCC ATG AAG      432
         Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Met Lys
                 130                 135                 140

         AGG TAT TTC CAG GGC ATC CAT GTC TAC CTG AAA GAG AAG GGA TAT AGT      480
 45      Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser
         145                 150                 155                 160

         GAC TGC GCC TGG GAA ATT GTC AGA CTG GAA ATC ATG AGA TCC TTG TCT      528
         Asp Cys Ala Trp Glu Ile Val Arg Leu Glu Ile Met Arg Ser Leu Ser
                         165                 170                 175
```

```
TCA TCA ACC AGC TTG CAC AAA AGG TTA AGA ATG ATG GAT GGA GAC CTG        576
Ser Ser Thr Ser Leu His Lys Arg Leu Arg Met Met Asp Gly Asp Leu
            180                 185                 190
```

```
AGC TCA CCT TGA                                                        588
Ser Ser Pro
        195
```

(2) INFORMATION FOR SEQ ID NO:32:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 195 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
                 of SEQ ID NO:31 (HuIFNtau3)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
 1               5                  10                  15

Gly Pro Gly Gly Ser Leu Arg Cys Asp Leu Ser Gln Asn His Val Leu
                20              25                  30

Val Gly Ser Gln Asn Leu Arg Leu Leu Gly Gln Met Arg Arg Leu Ser
            35                  40                  45

Leu Arg Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Phe Pro Gln Glu
        50                  55                  60

Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu
    65                  70                  75                  80

His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser
                85                  90                  95

Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu
                100                 105                 110

His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val Thr Gly
            115                 120                 125

Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Met Lys
    130                 135                 140

Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser
145                 150                 155                 160

Asp Cys Ala Trp Glu Ile Val Arg Leu Glu Ile Met Arg Ser Leu Ser
                165                 170                 175

Ser Ser Thr Ser Leu His Lys Arg Leu Arg Met Met Asp Gly Asp Leu
                180                 185                 190

Ser Ser Pro
        195
```

(2) INFORMATION FOR SEQ ID NO:33:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 518 base pairs
        (B) TYPE: nucleic acid

```
        (C)  STRANDEDNESS: double
        (D)  TOPOLOGY: linear

    (ii)  MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv)  ANTI-SENSE: NO

    (vi)  ORIGINAL SOURCE:
        (C)  INDIVIDUAL ISOLATE: HuIFNtau3, mature no leader sequence

    (ix)  FEATURE:
        (A)  NAME/KEY: CDS
        (B)  LOCATION: 1..518

    (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:33:

TGT GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC AGC CAG AAC CTC AGG
48
Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Ser Gln Asn Leu Arg
 1               5                  10                  15

CTC CTG GGC CAA ATG AGG AGA CTC TCC CTT CGC TTC TGT CTG CAG GAC
96
Leu Leu Gly Gln Met Arg Arg Leu Ser Leu Arg Phe Cys Leu Gln Asp
                20                  25                  30

AGA AAA GAC TTC GCT TTC CCC CAG GAG ATG GTG GAG GGT GGC CAG CTC      144
Arg Lys Asp Phe Ala Phe Pro Gln Glu Met Val Glu Gly Gly Gln Leu
            35                  40                  45

CAG GAG GCC CAG GCC ATC TCT GTG CTC CAC GAG ATG CTC CAG CAG AGC      192
Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
        50                  55                  60

TTC AAC CTC TTC CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC      240
Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
 65                  70                  75                  80

CTC CTG GAG CAG CTC CGC ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG      288
Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asp Leu
                85                  90                  95

GAT GCC TGC CTG GGG CAG GTG ACG GGA GAG GAA GAC TCT GCC CTG GGA      336
Asp Ala Cys Leu Gly Gln Val Thr Gly Glu Glu Asp Ser Ala Leu Gly
                100                 105                 110

AGA ACG GGC CCC ACC CTG GCC ATG AAG AGG TAT TTC CAG GGC ATC CAT      384
Arg Thr Gly Pro Thr Leu Ala Met Lys Arg Tyr Phe Gln Gly Ile His
            115                 120                 125

GTC TAC CTG AAA GAG AAG GGA TAT AGT GAC TGC GCC TGG GAA ATT GTC      432
Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
        130                 135                 140

AGA CTG GAA ATC ATG AGA TCC TTG TCT TCA TCA ACC AGC TTG CAC AAA      480
Arg Leu Glu Ile Met Arg Ser Leu Ser Ser Ser Thr Ser Leu His Lys
145                 150                 155                 160

AGG TTA AGA ATG ATG GAT GGA GAC CTG AGC TCA CCT TG                   518
Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
                165                 170

(2) INFORMATION FOR SEQ ID NO:34:
```

```
        (i)  SEQUENCE CHARACTERISTICS:
             (A)  LENGTH: 172 amino acids
             (B)  TYPE: amino acid
             (D)  TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

   Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Ser Gln Asn Leu Arg
    1               5                  10                  15

   Leu Leu Gly Gln Met Arg Arg Leu Ser Leu Arg Phe Cys Leu Gln Asp
                20                  25                  30

   Arg Lys Asp Phe Ala Phe Pro Gln Glu Met Val Glu Gly Gly Gln Leu
            35                  40                  45

   Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
            50                  55                  60

   Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
    65                  70                  75                  80

   Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asp Leu
                85                  90                  95

   Asp Ala Cys Leu Gly Gln Val Thr Gly Glu Glu Asp Ser Ala Leu Gly
                100                 105                 110

   Arg Thr Gly Pro Thr Leu Ala Met Lys Arg Tyr Phe Gln Gly Ile His
            115                 120                 125

   Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
        130                 135                 140

   Arg Leu Glu Ile Met Arg Ser Leu Ser Ser Ser Thr Ser Leu His Lys
    145                 150                 155                 160

   Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
                165                 170

   (2)  INFORMATION FOR SEQ ID NO:35:

        (i)  SEQUENCE CHARACTERISTICS:
             (A)  LENGTH: 37 amino acids
             (B)  TYPE: amino acid
             (D)  TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (vi) ORIGINAL SOURCE:
             (C)  INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                                      1-37 of SEQ    NO:33

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35

   Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Ser Gln Asn Leu Arg
    1               5                  10                  15

   Leu Leu Gly Gln Met Arg Arg Leu Ser Leu Arg Phe Cys Leu Gln Asp
                20                  25                  30

   Arg Lys Asp Phe Ala
            35

   (2)  INFORMATION FOR SEQ ID NO:36:
```

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 31 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
    (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment 34-64 of SEQ ID NO:33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Lys Asp Phe Ala Phe Pro Gln Glu Met Val Glu Gly Gly Gln Leu Gln
 1               5                  10                  15
Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 31 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
    (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment 62-92 of SEQ ID NO:33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp
 1               5                  10                  15
Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 33 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
    (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment 90-122 of SEQ ID NO:33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val Thr Gly
 1               5                  10                  15
Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Met Lys
            20                  25                  30
Arg
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                                119-150 of SEQ ID NO:33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ala Met Lys Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys
1   5   10   15

Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val Arg Leu Glu Ile Met Arg
            20   25   30

(2) INFORMATION FOR SEQ ID NO:40:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 34 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (vi) ORIGINAL SOURCE:
                (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                                        139-172 of SEQ ID NO:33

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Cys Ala Trp Glu Ile Val Arg Leu Glu Ile Met Arg Ser Leu Ser Ser
1   5   10   15

Ser Thr Ser Leu His Lys Arg Leu Arg Met Met Asp Gly Asp Leu Ser
            20   25   30

Ser Pro

(2) INFORMATION FOR SEQ ID NO:41:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 23 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (vi) ORIGINAL SOURCE:
                (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                                        1-23 of SEQ ID NO:32

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
1   5   10   15

Gly Pro Gly Gly Ser Leu Arg
            20

(2) INFORMATION FOR SEQ ID NO:42:

```
          (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 23 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

         (ii) MOLECULE TYPE: protein

         (vi) ORIGINAL SOURCE:
                (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                      1-23 of SEQ ID NO:11

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
 1           5               10                  15

Gly Pro Gly Gly Ser Leu Gly
            20

(2) INFORMATION FOR SEQ ID NO:43:

          (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 519 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear

         (ii) MOLECULE TYPE: DNA (genomic)

        (iii) HYPOTHETICAL: NO

         (iv) ANTI-SENSE: NO

         (vi) ORIGINAL SOURCE:
                (C) INDIVIDUAL ISOLATE: HuIFNtau1 genomic-derived
                      DNA coding sequence, without leader seq.

         (ix) FEATURE:
                (A) NAME/KEY: CDS
                (B) LOCATION: 1..519

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TGT GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC AGG AAG AAC CTC AGG
48
Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
 1           5               10                  15

CTC CTG GAC GAA ATG AGG AGA CTC TCC CCT CGC TTT TGT CTG CAG GAC
96
Leu Leu Asp Glu Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp
            20              25              30

AGA AAA GAC TTC GCT TTA CCC CAG GAA ATG GTG GAG GGC GGC CAG CTC   144
Arg Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu
            35              40              45

CAG GAG GCC CAG GCC ATC TCT GTG CTC CAT GAG ATG CTC CAG CAG AGC   192
Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
      50              55              60

TTC AAC CTC TTC CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC   240
Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
   65              70              75              80

CTC CTG GAG CAG CTC CGC ACT GGA CTC CAT CAG CAG CTG GAC AAC CTG   288
Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu
```

106

```
                          85                    90                    95
         GAT GCC TGC CTG GGG CAG GTG ATG GGA GAG GAA GAC TCT GCC CTG GGA     336
         Asp Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly
                     100                   105                   110

  5      AGG ACG GGC CCC ACC CTG GCT CTG AAG AGG TAC TTC CAG GGC ATC CAT     384
         Arg Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His
                     115                   120                   125

         GTC TAC CTG AAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ACC GTC     432
         Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val
  10                 130                   135                   140

         AGA CTG GAA ATC ATG AGA TCC TTC TCT TCA TTA ATC AGC TTG CAA GAA     480
         Arg Leu Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu
         145                   150                   155                   160

  15     AGG TTA AGA ATG ATG GAT GGA GAC CTG AGC TCA CCT TGA                 519
         Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
                     165                   170
```

        (2) INFORMATION FOR SEQ ID NO:44:

              (i) SEQUENCE CHARACTERISTICS:
                   (A) LENGTH: 172 amino acids
 20   (B) TYPE: amino acid
                   (D) TOPOLOGY: linear

              (ii) MOLECULE TYPE: protein

              (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

 25   Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
        1   5   10   15

        Leu Leu Asp Glu Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp
                    20   25   30

        Arg Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu
                    35   40   45

 30   Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
            50   55   60

        Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
        65   70   75   80

 35   Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu
                    85   90   95

        Asp Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly
                    100   105   110

        Arg Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His
                    115   120   125

 40   Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val
                    130   135   140

        Arg Leu Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu
        145   150   155   160

        Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
 45   165   170

(2) INFORMATION FOR SEQ ID NO:45:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
5   (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

10   (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Bin 12, A40068, Acc. gi 108955, Bovine
            TP-1 (clone bTP509) (aa 1-37)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

15   Cys Tyr Leu Ser Glu Asp His Met Leu Gly Ala Arg Glu Asn Leu Arg
    1   5   10   15

    Leu Leu Ala Arg Met Asn Arg Leu Ser Pro His Pro Cys Leu Gln Asp
            20   25   30

    Arg Lys Asp Phe Gly
           35

20   (2) INFORMATION FOR SEQ ID NO:46:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
25   (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

30   (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Bin 13, BovTPH1Bcds1, Acc. gi 163767,
            Bovine TP-1 (aa 1-37)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

    Cys Tyr Leu Ser Glu His His Ile Leu Gly Pro Arg Gln Asn Leu Ser
    1   5   10   15

35   Leu Leu Ala Arg Met Asn Arg Leu Ser Pro His Pro Cys Leu Gln Asp
            20   25   30

    Arg Lys Asp Phe Gly
           35

(2) INFORMATION FOR SEQ ID NO:47:

40   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
    (C) INDIVIDUAL ISOLATE: Bin 14, BovTPH1Ccds1, Acc. gi
        163769, Bovine TP-1 (aa 1-37)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Cys Tyr Leu Ser Glu His His Met Leu Gly Ala Arg Gln Asn Leu Arg
1   5   10   15

Leu Leu Ala Arg Met Asn Arg Leu Ser Pro His Pro Cys Leu Gln Asp
        20   25   30

Arg Lys Asp Phe Gly
        35

(2) INFORMATION FOR SEQ ID NO:48:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Bin 15, A39505, Bovine TP-1 (clone
        bTP4) (aa 1-37)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Cys Tyr Leu Ser Glu Asn His Met Leu Gly Ala Arg Glu Asn Leu Arg
1   5   10   15

Leu Leu Ala Arg Met Asn Arg Leu Ser Pro His Pro Cys Leu Gln Asp
        20   25   30

Arg Lys Asp Phe Gly
        35

(2) INFORMATION FOR SEQ ID NO:49:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:

            (C) INDIVIDUAL ISOLATE: Bin 16, OATP1P5cds1, Acc. gi 1412,
                 Ovine TPp5 (aa 1-37)

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

            Cys Tyr Leu Ser Gln Arg Leu Met Leu Asp Ala Lys Glu Asn Leu Lys
            1   5   10   15

            Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
                        20   25   30

            Arg Lys Asp Phe Gly
                        35

    (2) INFORMATION FOR SEQ ID NO:50:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 37 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

        (vi) ORIGINAL SOURCE:
             (C) INDIVIDUAL ISOLATE: Bin 17, OATP1P3cds1, Acc. gi 1410,
                 Ovine TPp3 (aa 1-37)

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

            Cys Tyr Leu Ser Gln Arg Leu Met Leu Asp Ala Arg Glu Asn Leu Lys
            1   5   10   15

            Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
                        20   25   30

            Arg Lys Asp Phe Gly
                        35

    (2) INFORMATION FOR SEQ ID NO:51:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 37 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

        (vi) ORIGINAL SOURCE:
             (C) INDIVIDUAL ISOLATE: Bin 18, SHP010TPcds1, Acc. gi
                 165821, Ovine TP-1 (aa 1-37)

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

            Cys Tyr Leu Ser Gln Arg Leu Met Leu Asp Ala Arg Glu Asn Leu Lys

```
              1              5              10             15

              Leu Leu Glu Pro Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
                          20                    25              30

              Arg Lys Asp Phe Gly
                          35
```

(2) INFORMATION FOR SEQ ID NO:52:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Bin 19, SHP02TPcds1, Acc. gi 165823,
           Ovine TP-1 (aa 1-37)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

```
              Cys Tyr Leu Ser Gln Arg Leu Met Leu Asp Ala Arg Glu Asn Leu Arg
              1              5              10             15

              Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
                          20                    25              30

              Arg Lys Asp Phe Gly
                          35
```

(2) INFORMATION FOR SEQ ID NO:53:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Bin 21, GOTCTP1cds1, Acc. gi 164117,
           Capra hircus IFN tau (aa 1-37)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

```
              Cys Tyr Leu Ser Arg Arg Leu Met Leu Asp Ala Arg Glu Asn Leu Arg
              1              5              10             15

              Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Gln Gln Asp
                          20                    25              30

              Arg Lys Asp Phe Gly
                          35
```

(2) INFORMATION FOR SEQ ID NO:54:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 498 base pairs
        (B) TYPE: nucleic acid
5   (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA (genomic)

     (iii) HYPOTHETICAL: NO

     (iv) ANTI-SENSE: NO

10   (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: huIFNtau/huIFNalpha hybrid
          (1-28/29-167)

     (ix) FEATURE:
        (A) NAME/KEY: CDS
15   (B) LOCATION: 1..498

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

```
TGT GAC TTG TCT CAA AAC CAC GTT TTG GTT GGT AGA AAG AAC TTA AGA
48
Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
  1               5                  10                  15

CTA CTA GAC GAA ATG AGA CGT CTA TCT CCA CGC TTC TGC CTG AAG GAC
96
Leu Leu Asp Glu Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Lys Asp
             20                  25                  30

AGA TAT GAT TTC GGA TTC CCC CAG GAG GTG TTT GAT GGC AAC CAG TTC        144
Arg Tyr Asp Phe Gly Phe Pro Gln Glu Val Phe Asp Gly Asn Gln Phe
         35                  40                  45

CAG AAG GCT CAA GCC ATC TCT GCC TTC CAT GAG ATG ATC CAG CAG ACC        192
Gln Lys Ala Gln Ala Ile Ser Ala Phe His Glu Met Ile Gln Gln Thr
     50                  55                  60

TTC AAT CTC TTC AGC ACA AAG GAT TCA TCT GCT GCT TGG GAT GAG ACC        240
Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 65                  70                  75                  80

CTC CTA GAC AAA TTC TAC ATT GAA CTT TTC CAG CAA CTG AAT GAC CTA        288
Leu Leu Asp Lys Phe Tyr Ile Glu Leu Phe Gln Gln Leu Asn Asp Leu
                 85                  90                  95

GAA GCC TGT GTG ACA CAG GAG GTT GGG GTG GAA GAG ATT GCC CTG ATG        336
Glu Ala Cys Val Thr Gln Glu Val Gly Val Glu Glu Ile Ala Leu Met
            100                 105                 110

AAT GAG GAC TCC ATC CTG GCT GTG AGG AAA TAC TTT CAA AGA ATC ACT        384
Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
            115                 120                 125

CTT TAT CTG ATG GGG AAG AAA TAC AGC CCT TGT GCC TGG GAG GTT GTC        432
Leu Tyr Leu Met Gly Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
        130                 135                 140

AGA GCA GAA ATC ATG AGA TCC TTC TCT TTT TCA ACA AAC TTG CAA AAA        480
Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser Thr Asn Leu Gln Lys
145                 150                 155                 160
```

```
GGA TTA AGA AGG AAG GAT
Gly Leu Arg Arg Lys Asp
            165
```
498

(2) INFORMATION FOR SEQ ID NO:55:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 166 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
 1           5               10              15

Leu Leu Asp Glu Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Lys Asp
            20              25              30

Arg Tyr Asp Phe Gly Phe Pro Gln Glu Val Phe Asp Gly Asn Gln Phe
            35              40              45

Gln Lys Ala Gln Ala Ile Ser Ala Phe His Glu Met Ile Gln Gln Thr
        50              55              60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 65              70              75              80

Leu Leu Asp Lys Phe Tyr Ile Glu Leu Phe Gln Gln Leu Asn Asp Leu
                85              90              95

Glu Ala Cys Val Thr Gln Glu Val Gly Val Glu Glu Ile Ala Leu Met
            100             105             110

Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
            115             120             125

Leu Tyr Leu Met Gly Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130             135             140

Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser Thr Asn Leu Gln Lys
145             150             155             160

Gly Leu Arg Arg Lys Asp
            165
```

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IT IS CLAIMED:

1. A chimeric nucleic acid molecule formed of

a 5' end segment that encodes the N-terminal amino acid sequence of an interferon-tau polypeptide, and

5   a 3' end segment that encodes the C-terminal amino acid sequence of a non-tau interferon type I polypeptide,

where the two segments are spliced in a region corresponding to the portion of a mature interferon polypeptide between about residues 8 and 37.

2. The chimeric nucleic acid molecule of claim 1, where said 5' end segment further includes

10   a leader sequence.

3. The chimeric nucleic acid molecule of claim 1, wherein said 5' end segment encodes an amino acid sequence contained in a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, and SEQ ID NO:54

15   and SEQ ID NO:55.

4. The chimeric nucleic acid molecule of claim 3, wherein said 5' end segment encodes an amino acid sequence contained in SEQ ID NO:5.

5. The chimeric nucleic acid molecule of claim 3, wherein said 5' end segment encodes an amino acid sequence contained in SEQ ID NO:15.

20   6. The chimeric nucleic acid molecule of claim 5, wherein said molecule has the sequence represented as SEQ ID NO:54.

7. The chimeric nucleic acid molecule of claim 1, wherein the two segments are spliced in a region corresponding to the portion of a mature interferon polypeptide between about residues 8 and 28.

25   8. The chimeric nucleic acid molecule of claim 7, wherein the two segments are spliced at a location corresponding to residue 22 of a mature interferon polypeptide.

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9. The chimeric nucleic acid molecule of claim 1, wherein the interferon-tau polypeptide is an ovine interferon-tau polypeptide and the non-tau interferon type I polypeptide is a human non-tau interferon type I polypeptide.

10. A hybrid interferon fusion polypeptide formed of

5   a first segment that contains the N-terminal amino acid sequence of an interferon-tau polypeptide, and

a second segment that contains the C-terminal amino acid sequence of a non-tau interferon type I polypeptide,

where the two segments are joined in the region of a mature interferon polypeptide between

10   about residues 8 and 37.

11. The interferon fusion polypeptide of claim 10, wherein said first segment has an amino acid sequence contained in a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53.

15   12. The interferon fusion polypeptide of claim 10, wherein said first segment has an amino acid sequence contained in SEQ ID NO:5.

13. The interferon fusion polypeptide of claim 10, wherein said first segment has an amino acid sequence contained in SEQ ID NO:15.

14. The interferon fusion polypeptide of claim 13, wherein said fusion polypeptide has the

20   sequence represented as SEQ ID NO:55.

15. The interferon fusion polypeptide of claim 10, wherein the two segments are joined in the region of a mature interferon polypeptide between about residues 8 and 28.

16. The interferon fusion polypeptide of claim 15, wherein the two segments are joined in the region of a mature interferon polypeptide between about residues 8 and 22.

25   17. The interferon fusion polypeptide of claim 16, wherein the two segments are joined in the region of a mature interferon polypeptide between about residues 8 and 16.

18. An expression vector comprising

(a) a nucleic acid containing an open reading frame that encodes the hybrid interferon fusion polypeptide of claim 10; and

(b) regulatory sequences effective to express said open reading frame in a host cell.

5   19. A method of recombinantly producing the hybrid interferon fusion polypeptide of claim 10, comprising

introducing into suitable host cells, a recombinant expression system containing an open reading frame (ORF) having a polynucleotide sequence which encodes the hybrid interferon fusion polypeptide of claim 10, where the vector is designed to express the ORF in said host, and

10   culturing said host under conditions resulting in the expression of the ORF sequence.

20. A method of inhibiting tumor cell growth, comprising

contacting the cells with the hybrid interferon fusion polypeptide of claim 10 at a concentration effective to inhibit growth of the tumor cells.

21. A method of inhibiting viral replication, comprising

15   contacting cells infected with a virus with the hybrid interferon fusion polypeptide of claim 10 at a concentration effective to inhibit viral replication within said cells.

22. A method of treating an autoimmune disease in a subject in need of such treatment, comprising

administering to said subject a pharmaceutically effective amount of hybrid interferon fusion

20   polypeptide of claim 10.

Fig. 1A

METOTP-1.SEQ4 -> Restriction Map

DNA sequence   540 b.p.   ATGTGGTACCAG ... CCGTAAGGTACC   linear

Fig. 1B

```
                    Fnu4H I
                    Bbv I
         BsmA I  Alu I       Xmn I              Mae III
Taq I    Pyu II           Msp I                 Hph I
         NsoB II          Hpa II      Fnu4H I    BstE II

TCGATTGTCTCCGCACAGCTGCCTGCAAGACCGGAAAGACTTCGGTCTGCCGCGCAGGAAATGGTTGAAGGTGACCAACTGC 160
AGCTAACAGAGGCGTGTCGACGGACGTTCTGGCCTTTCTGAAGCCAGACGGCGTCCTTTACCAACTTCCACTGGTTGACG

81       96     111                     129              148
   87    96     111                                      148
         97     114                                      149
         98
         98
```

```
         Msp I           Fnu4H I
         ScrF I          Bbv I
         Nci I           Pst I                 Fnu4H I
         Bcn I           Fnu4H I               Hae III
    Alu I Hpa II         Bbv I                 Gdi II
    HinD III Rsa I                 Mbo II      Eag I
                                              Eae I

AAAAAGACCAAGCTTTCCCGGTACTGTATGAAATGCTGCAGCAGTCTTTCAACCTGTTCTACACTGAACATCTTCGGCC 240
TTTTTCTGGTTCGAAAGGGCCATGACATACTTTACGACGTCGTCAGAAAGTTGGACAAGATGTGACTTGTGAAGAAGCCGG

    170    178     195                       232        236
    171    177     195                                  236
           177     196                                  236
           177     198                                  237
           178     198                                  238
```

Fig. 1C

```
                    Mae I
                    Xba I        HgiA I        Bsr I            Sau96 I
                    Mbo II       Bspl1286 I    AlwN I   Bsr I   Ava II
                    Bar I        ApaL I

GCTTGGGACACTACTCTTCTAGAACAACTGTGCACTGGTCTGCACGTGGTCTGCAACAGCAACTGGACCATCTGGACACTTGCCGTGGCCA 320
CGAACCCTGTGATGAGAAGATCTTGTTGACACGTGTCGTTGTCGTTGACCTGGTAGACCTGTGAACGGCACCGGT

                    254                270          286  291  294
                    255                270                    294
                         258           270
                         259                274                     Scrf I
                                                                    PflM I
                                                                    EcoR II
                                                                    BstN I
                                                                    Hae III
                                                                    Hae I
                                                                    Eae I
                                                                    Bal I
                                                           Sec I
                                                           Dsa I

                                                           312
                                                           312
                                                       315
                                                       315
                                                       31
                                                           316
                                                                    318
                                                                    318
                                                                    318
                                                                    318

                    Sau3A I
                    Mbo I
               Sau3A I
               Mbo I
               Dpn I
               Alw I
               Nla IV
```

Fig. 1D

```
Ple I

              Mbo II
              Bbv II
                            BstY I
         Mbo I                              BamH I
         Hph I    Hinf I    Mae III  Alw I  Mae III
                            Bsr I    Nla III  Pvu I  Dpn I   Mse I   Ssp I        Sec I
                                                                                  ScrF I
                                                                                  EcoR II
                                                                                  BstN I

GGTTATGGGTGAAGAGACTCTGAACTGGTAACATGGATCCGATCGTTACTGTTAAAAAATATTCCAGGTATCTACG 400
CCAATACCCACTTCTCTGAGACTTGACCATTGTACCTAGGCTAGCAATGACAATTTTTATAAAGGTCCCATAGATGC .

     328     337     345    354    363     374     380     387
         331     334     350       362    367                  387
                 334     357                                   387
                 337     357                                   387
                         357
                         357
                         358
                         358
                         358
                         358
                              363
                              363


                                    BstU I            Sau96 I
                                    Mlu I             Hae III
                                    Afl III           Sau96 I
                           Rsa I                      Nla IV
                           Spl I    SfaN I            Eco0109 I   Taq I
             HinP I                                   Bsp1286 I   Sal I
             Hha I                                    Ban II      HinC II
Pst I                                                Apa I   Tth111 I   Acc I
BspM I    Mae III

ACTACCTGCAGGAAAAAGGTTACTCTGACTGCGCTTGGGAAATCGTACGCGTTGAAATGATGCGGGCCCTGACTGTGTCG 480
TGATGGACGTCCTTTTTCCAATGAGACTGACGCGAACCCTTTAGCATGCGCAACTTTACTACGCCCGGGACTGACACAGC .
```

Fig. 1E

```
404
 406                 419              431      444                  459
                                     431      445
                                              447
                                              447
                                              448
     Mse I                   Mae III           Rsa I
     Hpa I                   Hph I   EcoR I    Nla IV
     HinC II                 BstE II           Kpn I
                                               Ban I
                                               Asp718
ACTACTCTGCAAAAACGGTTAACTAAAATGGGTGGTGACCTGAATTCTCCGTAAGGTACC 540
TGATGAGACGTTTTTGCCAATTGATTTTACCCACCACTGGACTTAAGAGGCATTCCATGG
     498              514      522             535
     498              514                      535
     499              515                      535
                                               535
                                               536

                             464    471    477
                             464           477
                             464           477
                             464           478
                             464
                             464
                             465
                             465
```

Fig. 2

```
                -23                                                       -9
                met ala phe val leu ser leu leu met ala leu val leu val ser
oIFNt     cccc  ATG GCC TTC GTG CTC TCT CTA CTG ATG GCC CTG GTG CTG GTC AGC
huIFNt1   cccc  ATG GCC TTC GTG CTC TCT CTA CTC ATG GCC CTG GTG CTG GTC AGC


     -8                                  -1 +1                            11
     tyr gly pro gly gly ser leu gly cys tyr leu ser arg lys leu met leu asp ala
     TAT GGC CCA GGA GGA TCT CTG GGT TGT TAC CTA TCT CGG AAA CTC ATG CTG GAT GCC
     TAC GGC CCA GGA GGA TCC CTG GGT TGT GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC
                                         asp         gln asn his val     val gly


      12                                                                   30
      arg glu asn leu lys leu leu asp arg met asn arg leu ser pro his ser cys leu
      AGG GAG AAC CTC AAG CTC CTG GAC CGA ATG AAC AGA CTC TCC CCT CAT TCC TGT CTG
      AGG AAG AAC CTC AGG CTC CTG GAC GAA ATG AGG AGA CTC TCC CCT CGC TTT TGT CTG
          lys         arg             glu     arg                 arg phe


      31                                                                   49
      gln asp arg lys asp phe gly leu pro gln glu met val glu gly arg gln leu gln
      CAG GAC AGA AAA GAC TTT GGT CTT CCC CAG GAG ATG GTG GAG GGC GAC CAG CTC CAG
      CAG GAC AGA AAA GAC TTC GCT TTA CCC CAG GAA ATG GTG GAG GGC GGC CAG CTC CAG
                              ala                                 gly


      50                                                                   68
      lys asp gln ala phe pro val leu tyr glu met leu gln gln ser phe asn leu phe
      AAG GAC CAG GCC TTC CCT GTG CTC TAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC
      GAG GCC CAG GCC ATC TCT GTG CTC CAT GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC
      glu ala         ile ser         his


      69                                                                   87
      tyr thr glu his ser ser ala ala trp asp thr thr leu leu glu gln leu cys thr
      TAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC TGC ACT
      CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC ACT
      his                                                                 arg


      88                                                                   106
      gly leu gln gln gln leu asp his leu asp thr cys arg gly gln val met gly glu
      GGA CTC CAA CAG CAG CTG GAC CAC CTG GAC ACC TGC AGG GGT CAA GTG ATG GGA GAG
      GGA CTC CAT CAG CAG CTG GAC AAC CTG GAT GCC TGC CTG GGG CAG GTG ATG GGA GAG
          his                     asn         ala     leu


      107                                                                  125
      glu asp ser glu leu gly asn met asp pro ile val thr val lys lys tyr phe gln
      GAA GAC TCT GAA CTG GGT AAC ATG GAC CCC ATT GTG ACC GTG AAG AAG TAC TTC CAG
      GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG GCT CTG AAG AGG TAC TTC CAG
                  ala         arg thr gly     thr leu ala leu     arg


      126                                                                  144
      gly ile tyr asp tyr leu gln glu lys gly tyr ser asp cys ala trp glu ile val
      GGC ATC TAT GAC TAC CTG CAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ATC GTC
      GGC ATC CAT GTC TAC CTG AAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ACC GTC
          his val         lys                                         thr


      145                                                                  163
      arg val glu met met arg ala leu thr val ser thr thr leu gln lys arg leu thr
      AGA GTC GAG ATG ATG AGA GCC CTC ACT GTA TCA ACC ACC TTG CAA AAA AGG TTA ACA
      AGA CTG GAA ATC ATG AGA TCC TTC TCT TCA TTA ATC AGC TTG CAA GAA AGG TTA AGA
          leu     ile         ser phe ser ser leu ile ser     glu         arg


      164               172
      lys met gly gly asp leu asn ser pro end
      AAG ATG GGT GGA GAT CTG AAC TCA CCT TGA
      ATG ATG GAT GGA GAC CTG AGC TCA CCT TGA
      met     asp         ser
```

Fig. 3
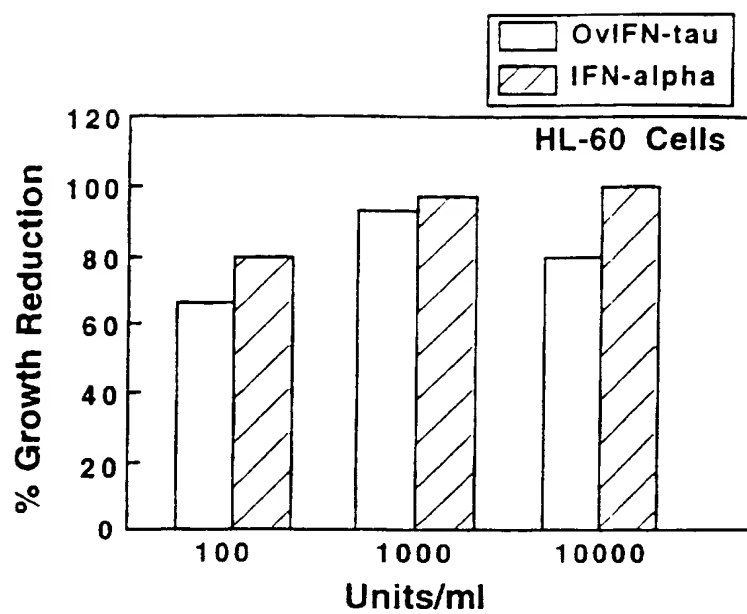
Fig. 4
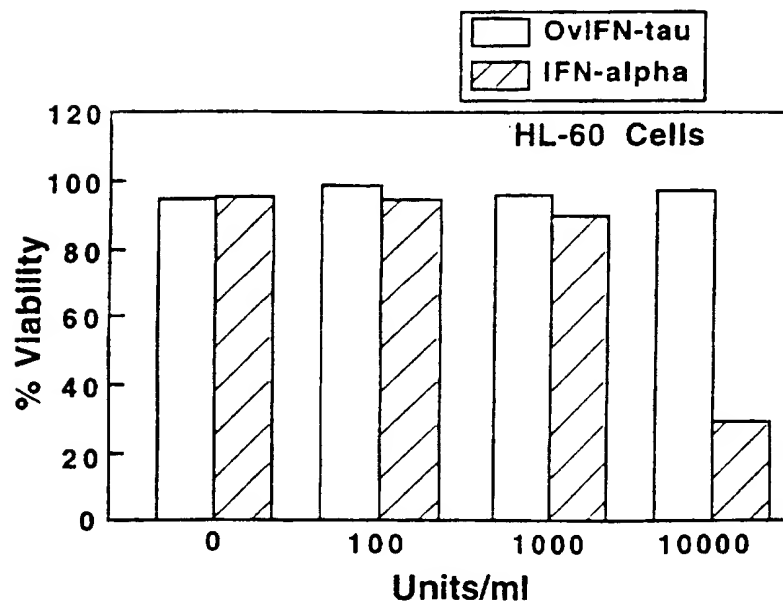
Fig. 5

Peptides	MW	HI*	Sequence
IFNt(1-37) (SEQ ID NO:5)	4465	-0.78	CYSLRKLMLDARENLKLLDRMNRLSPHSCLQDRKDFG
IFNt(34-64) (SEQ ID NO:6)	3610	-0.72	KDFGLPQEMVEGDQLQKDQAFPVLYEMLQQS
IFNt(62-92) (SEQ ID NO:7)	3586	-0.53	QQSFNLFYTEHSSAAWDTTLLEQLCTGLQQQ
IFNt(90-122) (SEQ ID NO:8)	3712	-0.86	QQQLDHLDTCRGQVMGEEDSELGNMDPIVTVKK
IFNt(119-150) (SEQ ID NO:9)	3948	-0.56	TVKKYFQGIYDYLQEKGYSDCAWEIVRVEMMR
IFNt(139-172) (SEQ ID NO:10)	3818	-0.11	CAWEIVRVEMMRALTVSTTLQKRLTKMGGDLNSP

*Hydropathic Index

Fig. 6
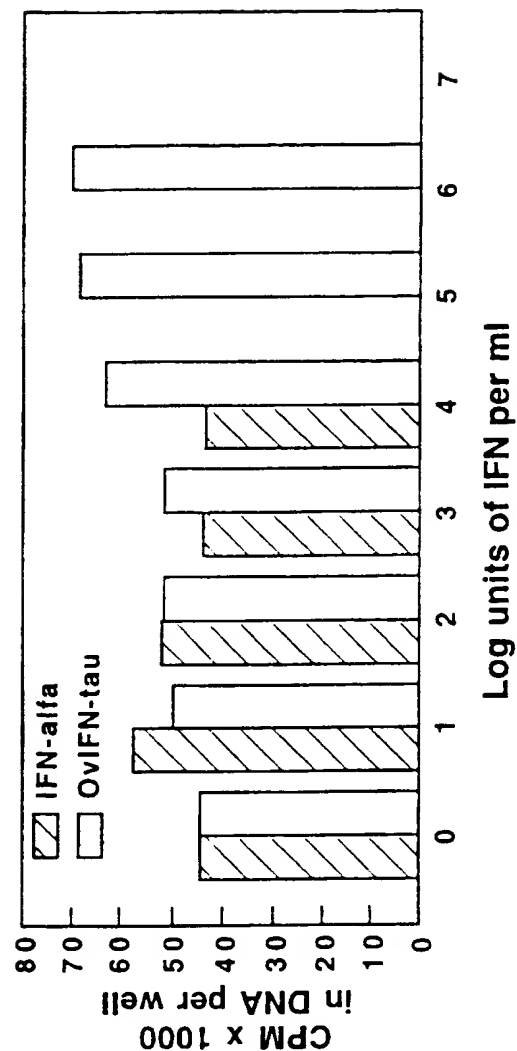
Fig. 8

```
1                                                                              80
CTGAGATGGGATCAGAGAACCTACCTGAAGGTTCCCCCTGACCCCATCTCAGCCAGCCCAGCAGCAGCCGCATCTTCCCC

81                                                                            140
  ATG GCC TTC GTG CTC TCT CTA CTG ATG GCC CTG GTG CTG GTC AGC TAT GGC CCA GGA GGA
  S1                                                                          S20
  Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr Gly Pro Gly Gly
141                                                                           200
  TCT CTG GGT TGT TAC CTA TCT CGG AAA CTC ATG CTG GAT GCC AGG GAG AAC CTC AAG CTC
  S21      1                                                                  17
  Ser Leu Gly Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys Leu
201                                                                           260
  CTG GAC CGA ATG AAC AGA CTC TCC CCT CAT TCC TGT CTG CAG GAC AGA AAA GAC TTT GGT
  18                                                                          37
  Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp Arg Lys Asp Phe Gly
261                                                                           320
  CTT CCC CAG GAG ATG GTG GAG GGC GAC CAG CTC CAG AAG GAC CAG GCC TTC CCT GTG CTC
  38                                                                          57
  Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu Gln Lys Asp Gln Ala Phe Pro Val Leu
321                                                                           380
  TAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC TAC ACA GAG CAC TCC TCT GCT GCC TGG
  58                                                                          77
  Tyr Glu Met Leu Gln Gln Ser Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp
381                                                                           440
  GAC ACC ACC CTC CTG GAG CAG CTC TGC ACT GGA CTC CAA CAG CAG CTG GAC CAC CTG GAC
  78                                                                          97
  Asp Thr Thr Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu Asp
441                                                                           500
  ACC TGC AGG GGT CAA GTG ATG GGA GAG GAA GAC TCT GAA CTG GGT AAC ATG GAC CCC ATT
  98                                                                          117
  Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly Asn Met Asp Pro Ile
501                                                                           560
  GTG ACC GTG AAG AAG TAC TTC CAG GGC ATC TAT GAC TAC CTG CAA GAG AAG GGA TAC AGC
  118                                                                         137
  Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr Asp Tyr Leu Gln Glu Lys Gly Tyr Ser
561                                                                           620
  GAC TGC GCC TGG GAA ATC GTC AGA GTC GAG ATG ATG AGA GCC CTC ACT GTA TCA ACC ACC
  138                                                                         157
  Asp Cys Ala Trp Glu Ile Val Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr
621                                                   666
  TTG CAA AAA AGG TTA ACA AAG ATG GGT GGA GAT CTG AAC TCA CCT TGA TGACTCTTGCCGACTA
  158                                                   172
  Leu Gln Lys Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro
                                                                               764
AGATGCCACATCAGCCTCCTACACCCGCCTGTGTTCATTTCAGAAGACTCTGATTTCTGCTCCAGCCACCAAATTCATTG

                                                                               844
AATTACTTTAGCTGATACTTTGTCAGTAGTAAAAAGCAAGTAGATATAAAAGTATTCAGCTGTAGGGGCATGAGTCCTGA

                                                                               924
AATGATGCCTTCCCTGATGTTATCTGTTGCTGATTTATTTATACCTTCTAGCATTTAACATACTTAAAATATTAGGAAAT

                     972
TTGTTAAGTTACATTTACATCTGTACATCATATTAAAATTTCTAAAACAAAAAAAAAA
```

Fig. 7

Fig. 9

Fig. 10

Fig. 11A

Control
OvIFN-tau
*(1-37)
*(139-172)

FIV-infected FET-1 Cells

RT activity: CPM x 1,000

Days

Fig. 11B

Control
OvIFN-tau
*(1-37)
*(139-172)

HIV-infected HPMC

RT activity: CPM x 1,000

Days

Fig. 12

Fig. 13

Fig. 14

Legend:

- ---■--- oIFNtau(1-37)   —△— oIFNtau(90-122)
- ● oIFNtau(34-64)   ---▲--- oIFNtau(119-150)
- ---●--- oIFNtau(62-92)   —□— oIFNtau(139-172)

Fig. 15

Fig. 16

Fig. 17

Fig. 18

```
          -23                                                        -9
          Met ala phe val leu ser leu leu met ala leu val leu val ser
oINFt    cccc ATG GCC TTC GTG CTC TCT CTA CTG ATG GCC CTG GTG CTG GTC AGC
LEXY.5   cccc                                    c
TOSHI.9    cc                                    c
TOSHI.10   cc                                    c


-8                                    -1  +1                          11
tyr gly pro gly gly ser leu gly cys tyr leu ser arg lys leu met leu asp ala
TAT GGC CCA GGA GGA TCT CTG GGT TGT TAC CTA TCT CGG AAA CTC ATG CTG GAT GCC
  c                     c           G       g   A   C   A   G     T   G
  c                     c    (---)G         g   A   C   A   G     T   G
  c       g             c    C G   G        g   A   C   A   G     T   G


12                                20                                  30
arg glu asn leu lys leu leu asp arg met asn arg leu ser pro his ser cys leu
AGG GAG AAC CTC AAG CTC CTG GAC CGA ATG AAC AGA CTC TCC CCT CAT TCC TGT CTG
    A           G           GA       GG                  GC  TT
    A           G            A       GG                  GC  TT
  C C           G            G A     GG              T   GC  T


31                                40                                  49
gln asp arg lys asp phe gly leu pro gln glu met val glu gly asp gln leu gln
CAG GAC AGA AAA GAC TTT GGT CTT CCC CAG GAG ATG GTG GAG GGC GAC CAG CTC CAG
                c   C   t   a           a               G
                c   C   t   a   TAG     a               G
                c   C   t   C                         t G
HuIFNt4                         !                     t G
HuIFNt5                         !                     t G
HuIFNt6                         !                       G
HuIFNt7                         !                   T AG     T


50                                60                                  68
lys asp gln ala phe pro val leu tyr glu met leu gln gln ser phe asn leu phe
AAG GAC CAG GCC TTC CCT GTG CTC TAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC
G   C           A   T           C T
G   C           A   T           C T
G   C           A   T           C
G   C           A   T           C
G   C           A   T           C
G   C           A   T           C   A
G   C           A T T           C T


69                                80                                  87
tyr thr glu his ser ser ala ala try asp thr thr leu leu glu gln leu cys thr
TAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC TGC ACT
C                                                                   C
C                                                                   C
C                                                                   C
C                                                                   C
C                                                                   C
C               G                                                   C
C   A           G                                   t               CT
```

Fig. 19A

```
88        90                                        100                    106
gly leu gln gln gln leu asp his leu asp thr cys arg gly gln val met gly glu
GGA CTC CAA CAG CAG CTG GAC CAC CTG GAC ACC TGC AGG GGT CAA GTG ATG GGA GAG
        T               A           t G     CT  g   g
        T               A           t G     CT  g   g
        T             t G           t G     CT  g   g       C
        T             t G           t G     CT  g   g       C
        T             t G           t G     CT  g   g       C
        T             t G             G     CT  g   g       C
        T             t G           t G   t CT  g   g T     CT


107         110                                     120        tyr phe gln 125
glu asp ser glu leu gly asn met asp pro ile val thr val lys lys tyr phe gln
GAA GAC TCT GAA CTG GGT AAC ATG GAC CCC ATT GTG ACC GTG AAG AAG TAC TTC CAG
        CC          a GG  C   G       CC C  G T C      G
        CC          a GG  C   G       CC C  G T C      G
        CC          a GA  C   G       CC C  G   A      G       t
        CC          a GG  C   G       CC C  G   A      C       t
        CC          a GG  C   G       CC C  G   A      C       t
        CC          a GG  C   G       CC C  G          GC
        CC          a GG  C   G       CC C  G          GC


126         130                              140            144
gly ile tyr asp tyr leu gln glu lys gly tyr ser asp cys ala trp glu ile val
GGC ATC TAT GAC TAC CTG CAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ATC GTC
        C   T           A                                           C
        C   T           A                                           C
        C   T           A               t   t                         t
        C   T           A               t   t               !
        C   T           A               t   !
        C   AT                              !
        C   T                   !


145         150                                     160        163
arg val glu met met arg ala leu thr val ser thr thr leu gln lys arg leu thr
AGA GTC GAG ATG ATG AGA GCC CTC ACT GTA TCA ACC ACC TTG CAA AAA AGG TTA ACA
    C G  a   C           T   T   T   TC  T   T   G         G               G
      g      a   C       T   T   T   TC  T   T   G         G               G
    C G  a   C           T   t g T   TC          G             C           G


164         172
lys met gly gly asp leu asn ser pro
AAG ATG GGT GGA GAT CTG AAC TCA CCT TGA
 T      A       c       G
 T      A       c       G
 T      A       c       G
```
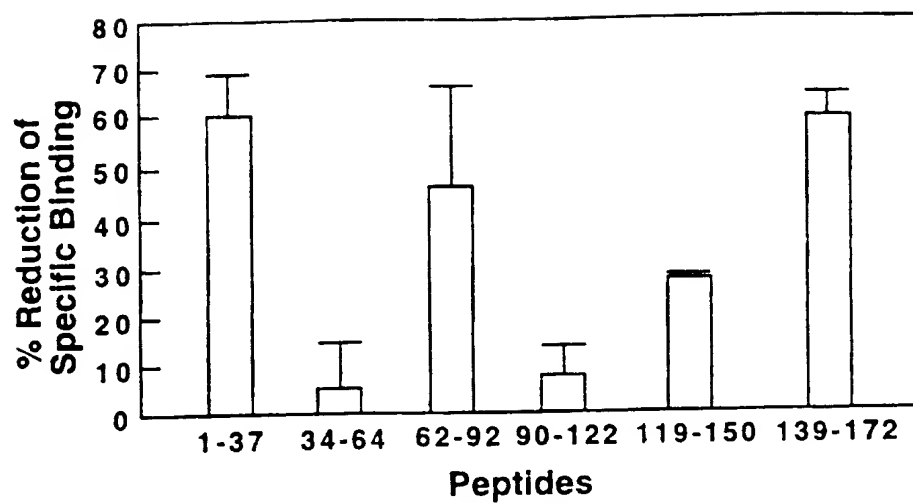
Fig. 19B

```
                                                                      -9
                    -23
                    Met ala phe val leu ser leu leu met ala leu val leu val ser
oTP-1
LEXY.5
TOSHI.9
TOSHI.10


-8                                -1  +1                                11
tyr gly pro gly gly ser leu gly cys tyr leu ser arg lys leu met leu asp ala
                                      asp         gln asn his val     val gly
                                  (---)asp        gln asn his val     val gly
                              arg     asp         gln asn his val     val gly


12                                20                                    30
arg glu asn leu lys leu leu asp arg met asn arg leu ser pro his ser cys leu
    lys         arg         glu     arg             arg phe
    lys         arg         gln     arg             arg phe
ser gln         arg     gly gln     arg         leu arg phe


31                                40                                    49
gln asp arg lys asp phe gly leu pro gln glu met val glu gly asp gln leu gln
                    ala                                 gly
                    ala         (Stop)                  gly
                    ala phe                             gly
Clone 21                          !                     gly
Clone 35                          !                     gly
Clone 15                          !                 val ser     phe
Clone 18                          !


50                                60                                    68
lys asp gln ala phe pro val leu tyr glu met leu gln gln ser phe asn leu phe
glu ala         ile ser         his
glu ala         ile ser         his
glu ala         ile ser         his
glu ala         ile ser         his
glu ala         ile ser         his
glu ala         ile ser         his lys
glu ala         ile ser         his


69                                80                                    87
tyr thr glu his ser ser ala ala try asp thr thr leu leu glu gln leu cys thr
his                                                                     arg
his                                                                     arg
his                                                                     arg
his                                                                     arg
his                                                                     arg
his                     arg                                             arg
his lys                 arg                                             leu
```
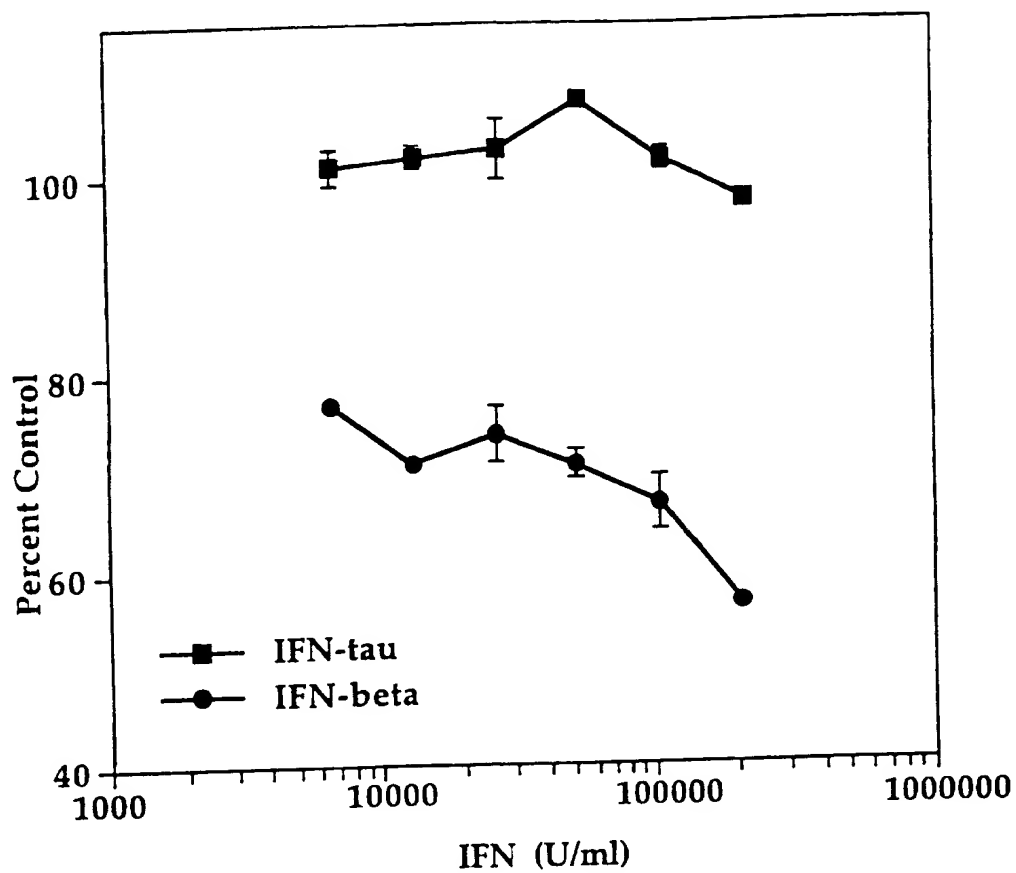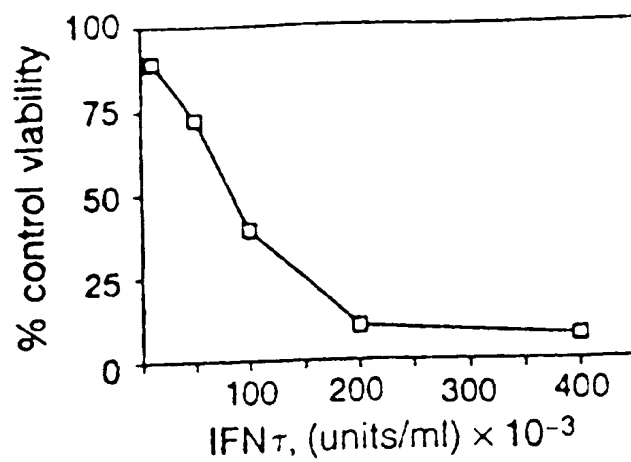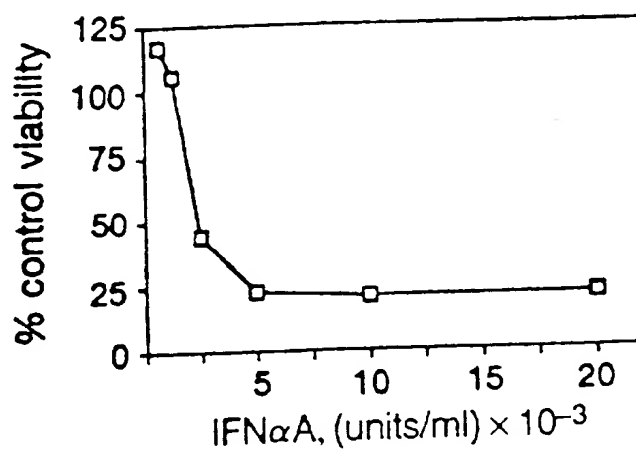
Fig. 20A

```
88        90                          100              106
gly leu gln gln gln leu asp his leu asp thr cys arg gly gln val met gly glu
        his             asn         ala     leu
        his             asn         ala     leu
        his             asp         ala     leu             thr
        his             asp         ala     leu             thr
        his             asp         ala     leu             thr
        his             asp         ala     leu             thr
        his             asp         ala     leu         leu thr

107     110                          120          125
glu asp ser glu leu gly asn met asp pro ile val thr val lys lys tyr phe gln
        ala         arg thr gly     thr leu ala leu     arg
        ala         arg thr gly     thr leu ala leu     arg
        ala         arg thr gly     thr leu ala met     arg
        ala         arg thr gly     thr leu ala met     thr
        ala         arg thr gly     thr leu ala met     thr
        ala         arg thr gly     thr leu ala         ser
        ala         arg thr gly     thr leu ala         ser

126         130                      140              144
gly ile tyr asp tyr leu gln glu lys gly tyr ser asp cys ala trp glu ile val
        his val         lys                                     thr
        his val         lys                                     thr
        his val         lys                                 !
        his val         lys                 !
        his val             !                   !
        his ile                 !
        his val
```

```
145             150                              160     163
arg val glu met met arg ala leu thr val ser thr thr leu gln lys arg leu thr
    leu     ile         ser phe ser ser leu ile ser     glu             arg
            ile         ser phe ser ser leu ile ser     glu             arg
    leu     ile         ser     ser ser         ser his                 arg
```

```
164                 172
lys met gly gly asp leu asn ser pro stop
met     asp             ser         stop
met     asp             ser         stop
met     asp             ser         stop
```
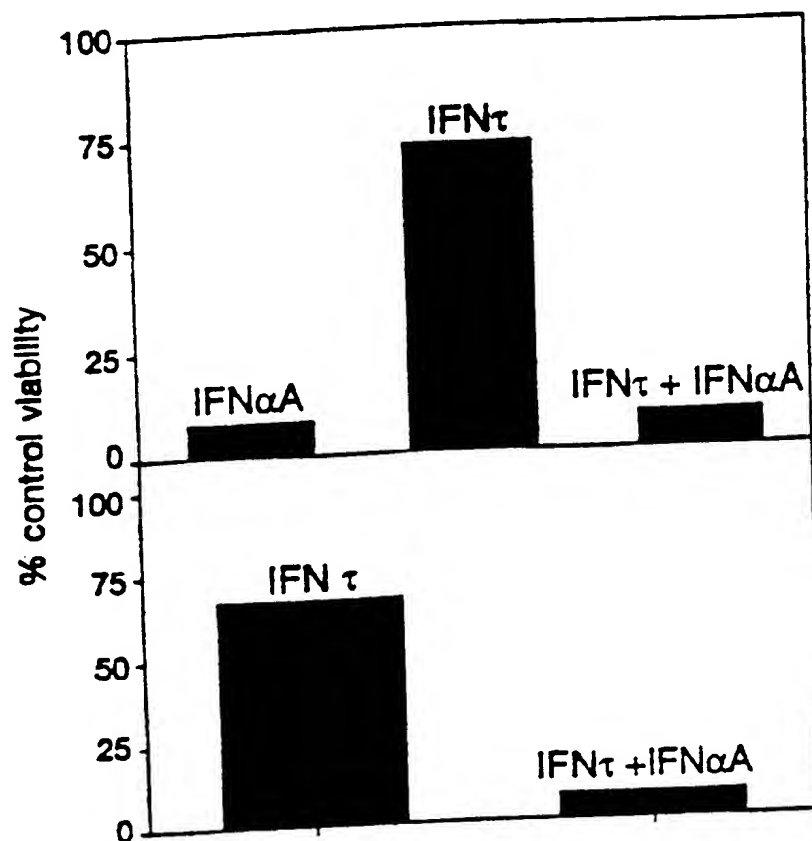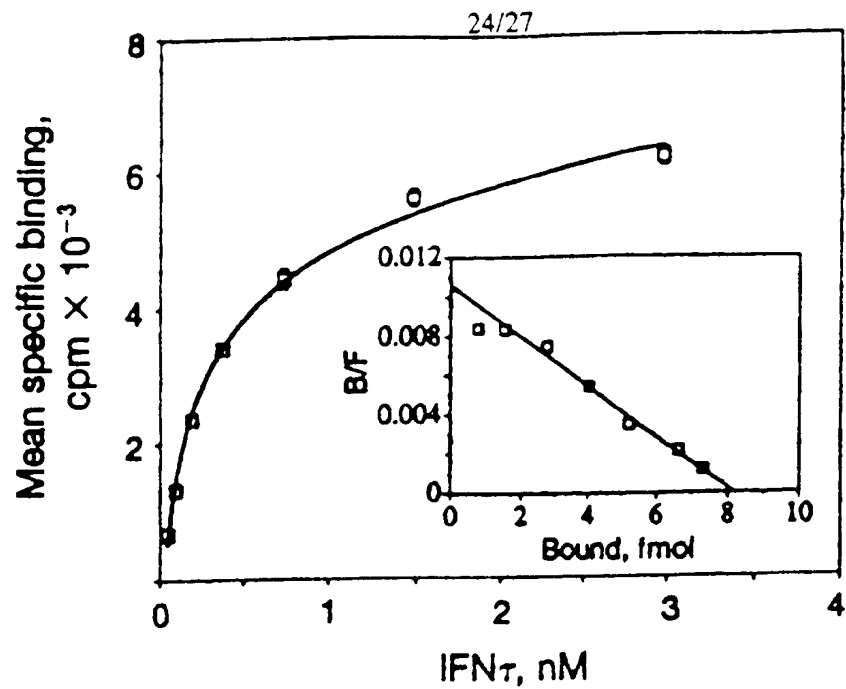
Fig. 20B

Fig. 21

Fig. 22A

Fig. 22B

Fig. 23A

Fig. 23B

Fig. 24A
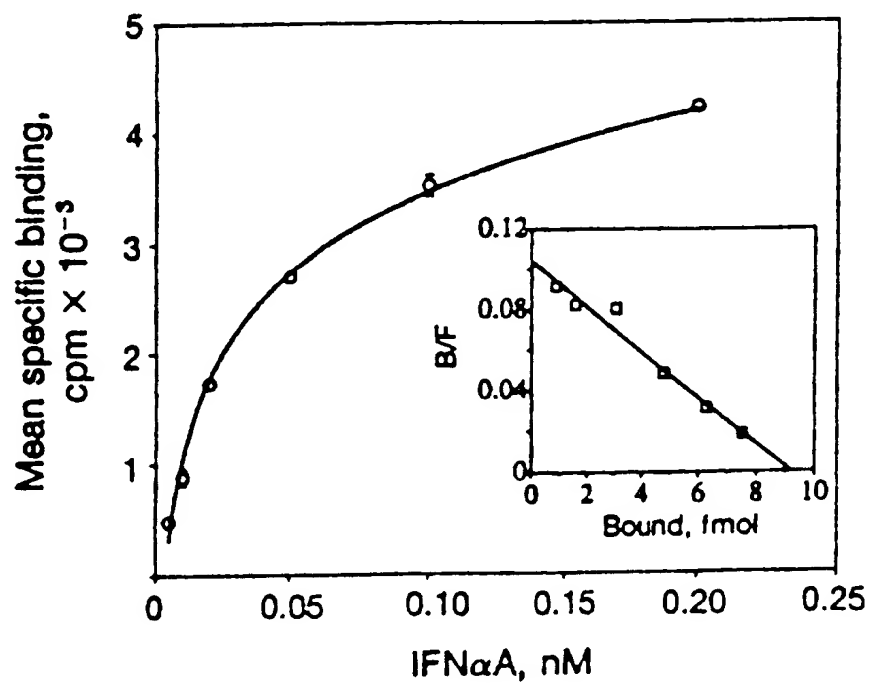
Fig. 24B
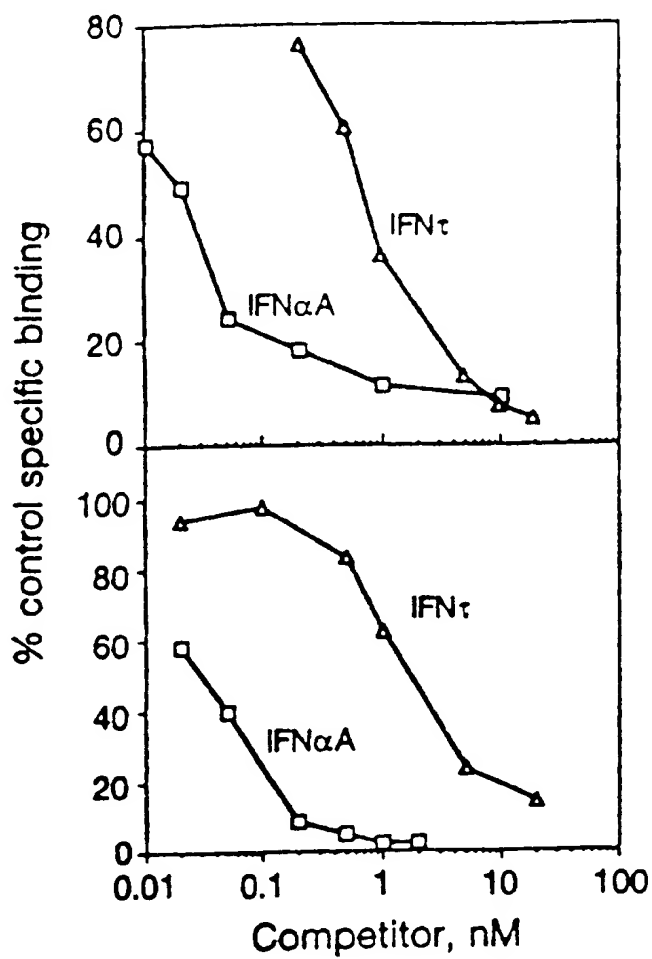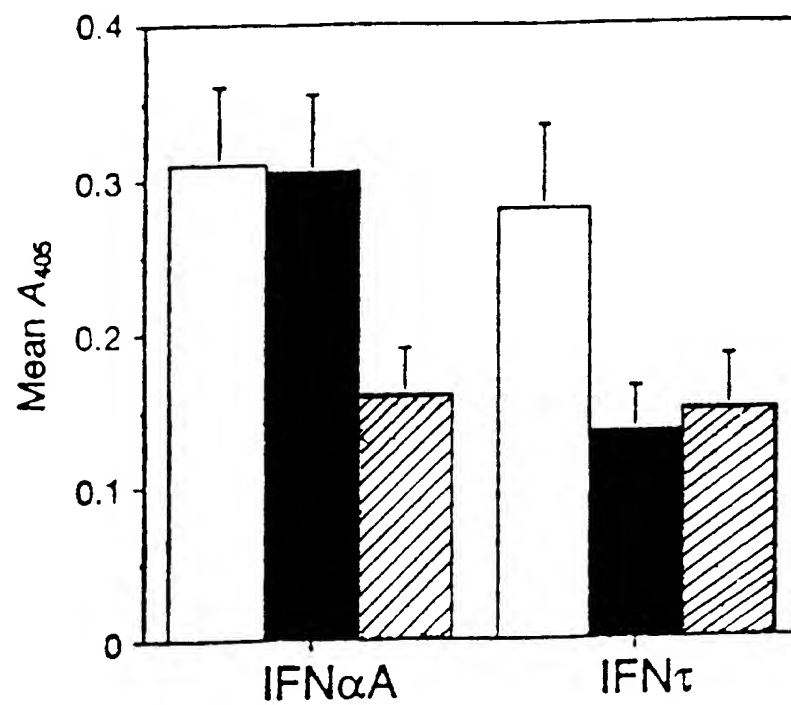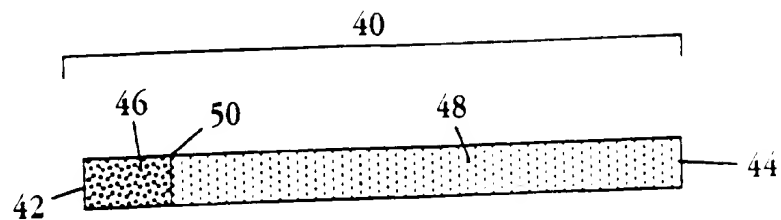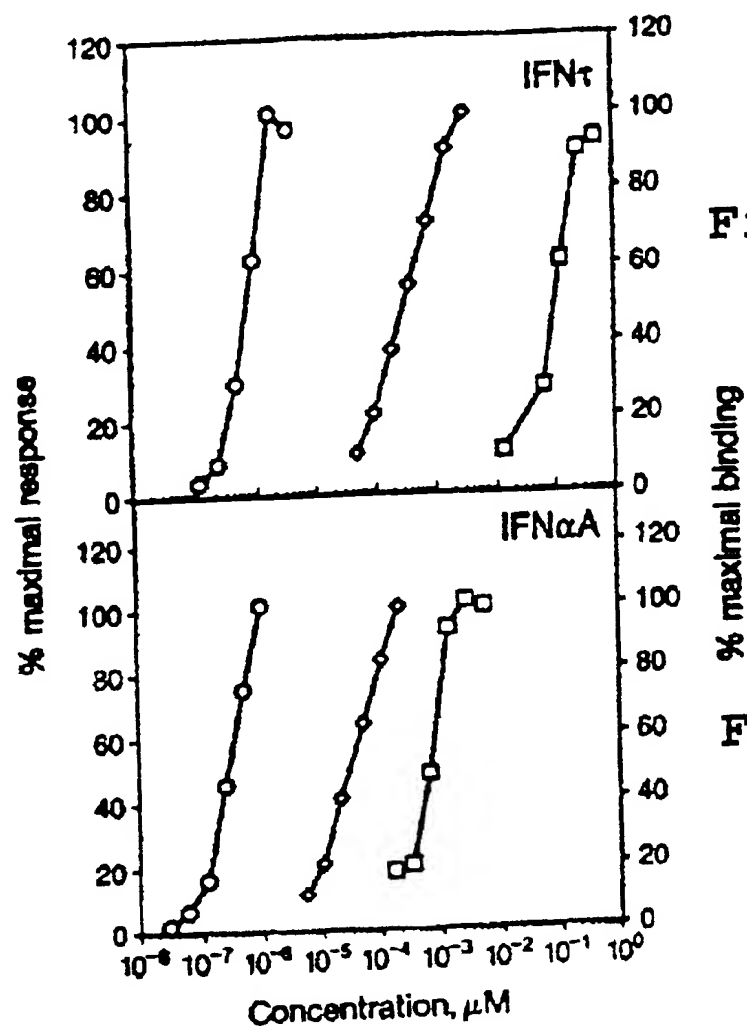
Fig. 25A

Fig. 25B

Fig. 26

Fig. 28

Fig. 27A

Fig. 27B

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/21   C12N15/62   C07K14/56   C07K14/19   A61K38/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 10313 A (UNIV FLORIDA ;WOMEN S RESEARCH INST (US)) 11 May 1994 see abstract; claims 47-52; figure SEQ.5 ---	1-5, 10-13, 18-21
A	JOURNAL OF IMMUNOLOGY, vol. 155, no. 5, 1 September 1995, pages 2747-2753, XP002010954 SOOS J M ET AL: "THE IFN PREGNANCY RECOGNITION HORMONE IFN-TAU BLOCKS BOTH DEVELOPMENT AND SUPERANTIGEN REACTIVATION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS WITHOUT ASSOCIATED TOXICITY" see the whole document ---	1-5, 10-13, 18-21
	-/--	

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

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'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

'&' document member of the same patent family

Date of the actual completion of the international search

21 August 1997

Date of mailing of the international search report

05.09.97

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Authorized officer

Gurdjian, D

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C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. BIOL. CHEM. (1994), 269(40), 24826-33 CODEN: JBCHA3;ISSN: 0021-9258, XP002038444 LI, JUNZHI ET AL: "Structure-function relationships in the interferon-.tau. (IFN-.tau.). Changes in receptor binding and in antiviral and antiproliferative activities resulting from site-directed mutagenesis performed near the carboxyl terminus" see the whole document ---	1-5, 10-13, 18-21
A	PROC NATL ACAD SCI U S A, DEC 19 1995, 92 (26) P12270-4, UNITED STATES, XP002038445 SUBRAMANIAM PS ET AL: "Differential recognition of the type I interferon receptor by interferons tau and alpha is responsible for their disparate cytotoxicities." see the whole document ---	1-5, 10-13, 18-21
A	EP 0 146 903 A (SCHERING CORP) 3 July 1985 cited in the application see example 5 ---	1-5, 10-13, 18-21
A	WO 83 02461 A (CETUS CORP) 21 July 1983 cited in the application see claims 1-11 -----	1-5, 10-13, 18-21

1

INTERNATIONAL SEARCH REPORT

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This Internauonal Search Report has not been established in respect of certain claims under Arucle 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark: Although claim 22 and claims 20,22 partially as far as they concern an in vivo method, are directed to a method of treatment of the human/ animal body, the search has been carried out and based on the alleged effects of that composition.

2. [ ] Claims Nos.:
   because they relate to parts of the International Applicauon that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This Internauonal Searching Authority found multiple invenuons in this international applicauon, as follows:

1. [ ] As all required additional search fees were umely paid by the applicant, this Internauonal Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were umely paid by the applicant, this Internauonal Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were umely paid by the applicant. Consequently, this Internauonal Search Report is restricted to the invenuon first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9410313 A	11-05-94	AU	5444994 A	24-05-94
		CN	1090510 A	10-08-94
		EP	0669981 A	06-09-95
		JP	8505047 T	04-06-96
EP 0146903 A	03-07-85	US	4892743 A	09-01-90
		JP	60222500 A	07-11-85
WO 8302461 A	21-07-83	AU	1155083 A	28-07-83
		CA	1210715 A	02-09-86
		EP	0098876 A	25-01-84
		US	4569908 A	11-02-86
		US	4758428 A	19-07-88